

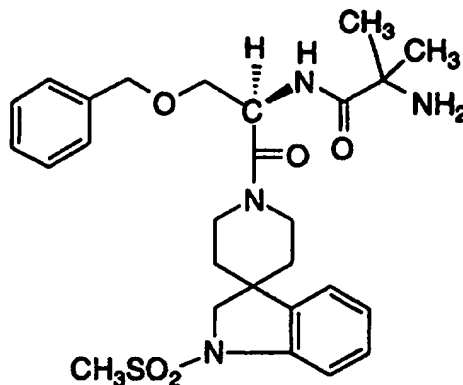


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(54) Title: PROCESS FOR THE PREPARATION OF A GROWTH HORMONE SECRETAGOGUE**(57) Abstract**

The present invention is directed to a novel process for the preparation of the compound N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide, and salts thereof, which has structure (1) and which has the ability to stimulate the release of natural or endogenous growth



(1)

hormone. This compound may be used to treat conditions which require the stimulation of growth hormone production or secretion such as in humans with a deficiency of natural growth hormone or in animals used for food or wool production where the stimulation of growth hormone will result in a larger, more productive animal.

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TITLE OF THE INVENTION**PROCESS FOR THE PREPARATION OF A GROWTH HORMONE
SECRETAGOGUE****5 BACKGROUND OF THE INVENTION**

Growth hormone, which is secreted from the pituitary, stimulates growth of all tissues of the body that are capable of growing. In addition, growth hormone is known to have the following basic effects on the metabolic processes of the body: (1) Increased rate of protein
10 synthesis in all cells of the body; (2) Decreased rate of carbohydrate utilization in cells of the body; (3) Increased mobilization of free fatty acids and use of fatty acids for energy. A deficiency in growth hormone secretion can result in various medical disorders, such as dwarfism.

Various ways are known to release growth hormone. For
15 example, chemicals such as arginine, L-3,4-dihydroxyphenylalanine (L-DOPA), glucagon, vasopressin, and insulin induced hypoglycemia, as well as activities such as sleep and exercise, indirectly cause growth hormone to be released from the pituitary by acting in some fashion on the hypothalamus perhaps either to decrease somatostatin secretion or
20 to increase the secretion of the known secretagogue growth hormone releasing factor (GRF) or an unknown endogenous growth hormone-releasing hormone or all of these.

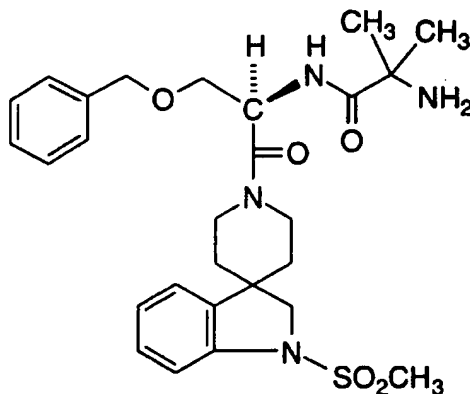
In cases where increased levels of growth hormone were desired, the problem was generally solved by providing exogenous
25 growth hormone or by administering GRF or a peptidal compound which stimulated growth hormone production and/or release. In either case the peptidyl nature of the compound necessitated that it be administered by injection. Initially the source of growth hormone was the extraction of the pituitary glands of cadavers. This resulted in a
30 very expensive product and carried with it the risk that a disease associated with the source of the pituitary gland could be transmitted to the recipient of the growth hormone. Recombinant growth hormone has become available which, while no longer carrying any risk of disease transmission, is still a very expensive product which

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must be given by injection or by a nasal spray. Other compounds have been developed which stimulate the release of endogenous growth hormone.

In particular, certain spiro compounds are disclosed in
5 PCT Patent Publication WO 94/13696 and Proc. Natl. Acad. Sci. USA, 92, 7001-7005 (July 1995) as being non-peptidal growth hormone secretagogues. These compounds have the ability to stimulate the release of natural or endogenous growth hormone and thus may be used to treat conditions which require the stimulation of
10 growth hormone production or secretion such as in humans with a deficiency of natural growth hormone or in animals used for food or wool production where the stimulation of growth hormone will result in a larger, more productive animal.

Among the preferred compounds disclosed therein is
15 spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethoxy)ethyl]-2-amino-2-methylpropanamide which has the structure:



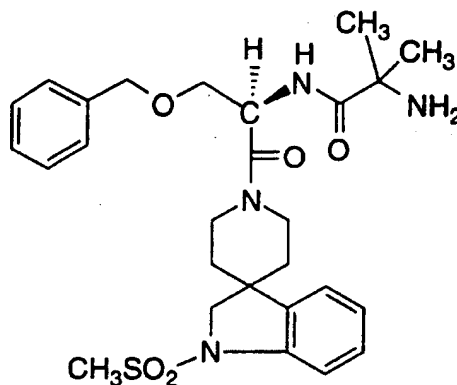
PCT Patent Publication WO 94/13696 discloses methods for preparing this compound (see Examples 18, 19 and 55). However,
20 the synthesis of the compound was accomplished by using the very expensive amino acid coupling agent EDC (\$1100/kg); the use of numerous equivalents of trifluoroacetic acid as the catalyst for the BOC group deprotections; extensive chromatographic purifications; and resulted in "gumming" of the final product.

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- The advantages of the present invention include: a 6-step high yielding non-isolation process providing material of $\geq 99.9\%$ purity; decreased expense through the use of DCC [\$40/kg] instead of EDC [\$1100/kg]; diminished environmental impact through the use of methanesulfonic acid instead of trifluoroacetic acid as the catalyst (as well as lesser equivalents of catalyst) in the deprotections; and ease of isolation of the final product.

SUMMARY OF THE INVENTION

- The instant invention is directed to a process for the preparation of the compound N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperidin]-1'-yl)carbonyl]-2-(phenylmethoxy)ethyl]-2-amino-2-methyl-propanamide which has the structure:



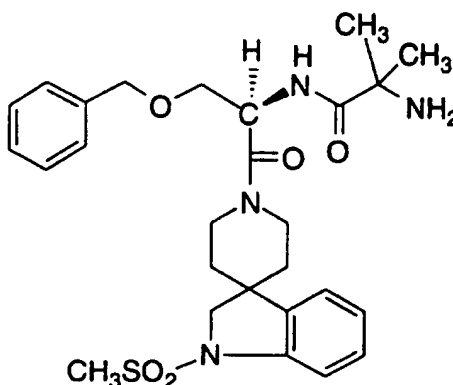
- and salts thereof, in particular, the methanesulfonate salt.

This compound has the ability to stimulate the release of natural or endogenous growth hormone and may be used to treat conditions which require the stimulation of growth hormone production or secretion such as in humans with a deficiency of natural growth hormone or in animals used for food or wool production where the stimulation of growth hormone will result in a larger, more productive animal.

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DESCRIPTION OF THE INVENTION

The present invention is directed to a novel process for the preparation of the compound N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethoxy)ethyl]-2-amino-2-methyl-propanamide which has the structure:



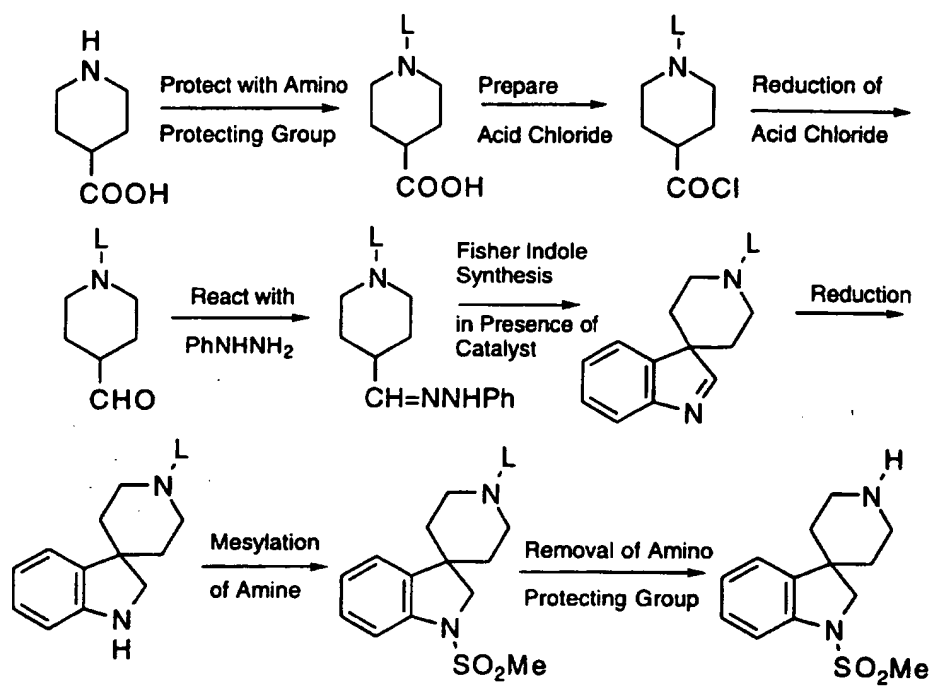
and salts thereof, in particular, the methanesulfonate salt.

The instant process provides the desired compound from readily available inexpensive and environmentally acceptable starting materials reagents and solvents. The process does not require the use any chromatographic purifications, and it is possible to produce the final product from the intermediate spiroindoline sulfonamide without isolation of any of the intermediates.

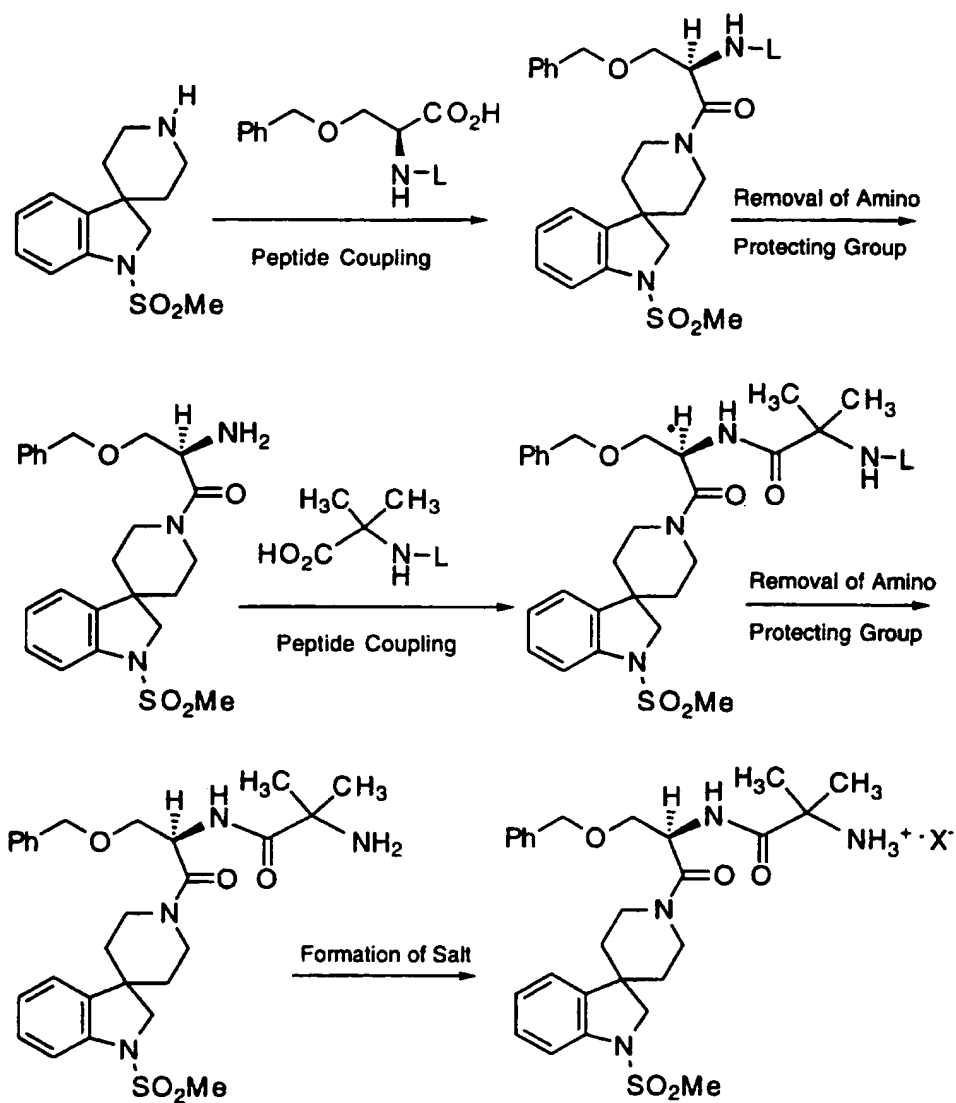
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The individual processes within the general process are summarized as follows:

SCHEME I



- 6 -

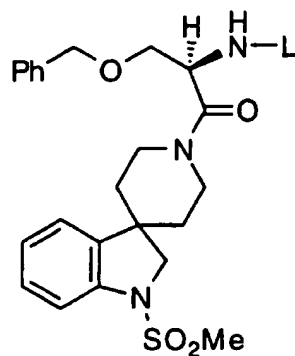
SCHEME I (CONT'D)

5

(wherein L is an appropriate amino protecting group and X⁻ is an appropriate counterion).

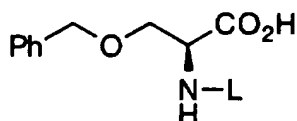
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Within this general process, a first process concerns the preparation of a compound of formula I:

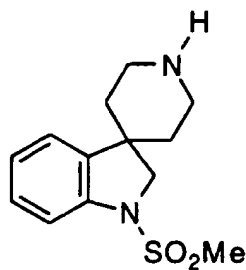


I

- 5 wherein L is an amino protecting group, by coupling an amino acid of the formula:



- 10 with a compound of the formula :



in the presence of an acid activating agent in an inert solvent in the presence of a catalytic agent, to give the compound of formula I.

- 8 -

Acid activating agents suitable for this process include: DCC, EDC, ECAC and BOP, in which the preferred acid activating agent is DCC (N,N'-dicyclohexylcarbodiimide).

Catalytic agents suitable for this process include: HOBT and
5 the like in which a preferred catalytic agent is HOBT (hydroxybenzotriazole).

Inert solvents appropriate for this processes include: acetonitrile; iso-propyl acetate; ethyl acetate; propionitrile; water; chlorinated hydrocarbons such as dichloromethane, chloroform, carbon
10 tetrachloride, dichloroethane, chlorobenzene, ortho-dichlorobenzene; benzene; toluene; xylenes; and the like; and mixtures thereof, in which the preferred solvent is either acetonitrile or isopropyl acetate and water.

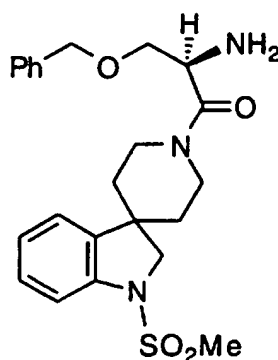
The preferred reaction temperature range is between -40 and 150°C, and the most preferred range is between 20 and 35°C.

Suitable amino protecting groups include: benzyl,
15 benzyloxymethyl, benzyloxycarbonyl (carbobenzyloxy), benzylsulfonyl, 2-bromo-ethyloxycarbonyl, t-butoxy-carbonyl, 2-chloro-benzyloxy-carbonyl, 2-chloroethyloxycarbonyl, di-t-amylloxycarbonyl, 9-fluoroenyl-methyloxycarbonyl, isopropoxycarbonyl, 4-methoxy-benzyloxycarbonyl,
20 4-nitrobenzyloxycarbonyl, 2-nitrophenyl-sulfonyl, phthaloyl, 2,2,2-trichloro-t-butyloxycarbonyl, trifluoroacetyl, triphenylmethane, allyloxycarbonyl, and vinyloxycarbonyl groups, and the like, in which the preferred ones include benzyloxycarbonyl (carbobenzyloxy), t-butoxy-carbonyl groups, and in which the most preferred one is the t-butoxy-
25 carbonyl group.

In the interest of efficiency, it is preferred that this coupling be conducted *in situ* without isolation of the compound of formula I following its preparation by the aforementioned process.

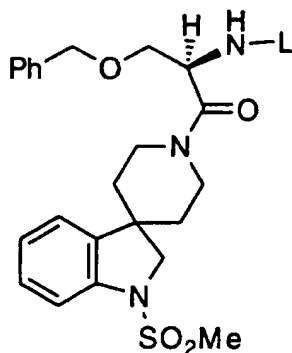
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Within this general process, a second process concerns the preparation of a compound of formula II:



II

5 which comprises reacting a compound of the formula I:



I

wherein L is an amino protecting group, with an amino deprotecting agent to give the compound of formula II.

- 10 Suitable amino protecting groups include: benzyl, benzyloxymethyl, benzyloxycarbonyl (carbobenzyloxy), benzylsulfonyl, 2-bromo-ethyloxycarbonyl, t-butoxy-carbonyl, 2-chloro-benzyloxy-carbonyl, 2-chloroethyloxycarbonyl, di-t-amylloxycarbonyl, 9-fluoroenyl-methyloxycarbonyl, isopropoxycarbonyl, 4-methoxy-benzyloxycarbonyl, 15 4-nitrobenzyloxycarbonyl, 2-nitrophenyl-sulfonyl, phthaloyl, 2,2,2-trichloro-t-butyloxycarbonyl, trifluoroacetyl, triphenylmethane, allyloxycarbonyl, and vinyloxycarbonyl groups, and the like, in which the preferred ones include benzyloxycarbonyl (carbobenzyloxy), t-butoxy-

- 10 -

carbonyl groups, and in which the most preferred one is the t-butoxy-carbonyl group.

In this process, the removal of the amino protecting group may be accomplished by use of an appropriate catalytic agent. Removal
5 of a t-butoxycarbonyl protecting group may be carried out in a solvent such as methanol, ethanol, methylene chloride, ethyl acetate, or isopropyl acetate, with a strong acid. Such strong acids include methanesulfonic acid, trifluoroacetic acid, hydrochloric acid, hydrogen
10 chloride gas, hydrogen bromide; hydrogen iodide; trifluoromethanesulfonic acid; camphorsulfonic acid; sulfuric acid; phosphoric acid; and an arylsulfonic acid, such as benzenesulfonic acid, p-toluenesulfonic acid, and p-chlorobenzene-sulfonic acid. Preferred catalytic agents include: trifluoroacetic acid; methanesulfonic acid; camphorsulfonic acid;
15 benzenesulfonic acid, p-toluenesulfonic acid; and p-chlorobenzene-sulfonic acid. The most preferred catalytic agent is methanesulfonic acid. The preferred solvent is methanol or ethanol, and the most preferred solvent is ethanol.

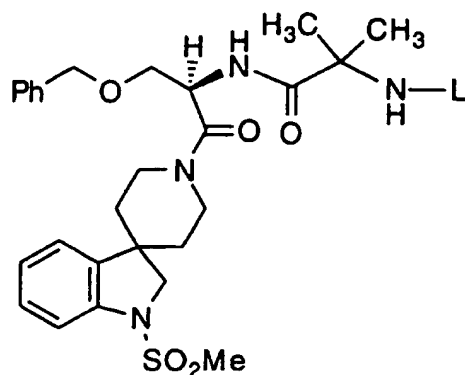
The preferred reaction temperature range is between -40 and 150°C, and the most preferred range is between 10 and 40°C.

20 Removal of a benzyloxycarbonyl (carbobenzyloxy) group may be achieved by a number of methods, for example, catalytic hydrogenation with hydrogen in the presence of a noble metal or its oxide such as palladium on activated carbon in a protic solvent such as ethanol. In cases where catalytic hydrogenation is contraindicated by the presence
25 of other potentially reactive functionality, the removal of benzyloxycarbonyl (carbobenzyloxy) group may also be achieved by treatment with a solution of hydrogen bromide in acetic acid, or by treatment with a mixture of TFA and dimethylsulfide.

In the interest of efficiency, it is preferred that this acid-catalyzed deprotection be conducted *in situ* without isolation of the
30 compound of formula II following its preparation by the aforementioned process.

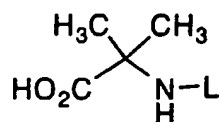
- 11 -

Within this general process, a third process concerns the preparation of a compound of formula III:

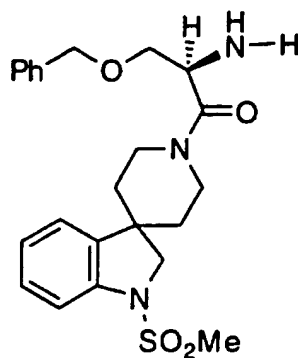


III

- 5 wherein L is an amino protecting group, by coupling an amino acid of the formula:



wherein L is an amino protecting group, with a compound of the formula II:



II

10

in the presence of an acid activating agent in an inert solvent in the presence of a catalytic agent, to give the compound of formula III.

Acid activating agents suitable for this process include:

- 12 -

DCC, EDC, ECAC and BOP, in which the preferred acid activating agent is DCC (N,N'-dicyclohexylcarbodiimide).

Catalytic agents suitable for this process include: HOBt and the like in which a preferred catalytic agent is HOBt

5 (hydroxybenzotriazole).

Inert solvents appropriate for this processes include: acetonitrile; isopropyl acetate; ethyl acetate; propionitrile; water; chlorinated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene, ortho-dichlorobenzene;
10 benzene; toluene; xylenes; and the like; and mixtures thereof, in which the preferred solvent is a mixture of iso-propyl acetate and water, preferably in a ratio of approximately 40:60 to 60:40 (by volume) and more preferably in a ratio of approximately 50:50 (by volume).

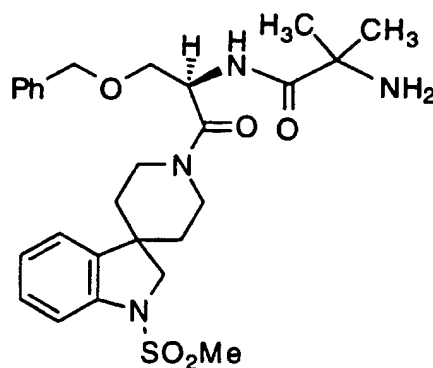
The preferred reaction temperature range is between -40 and
15 150°C, and the most preferred range is between 20 and 50°C.

Suitable amino protecting groups include: benzyl, benzyloxymethyl, benzyloxycarbonyl (carbobenzyloxy), benzylsulfonyl, 2-bromo-ethyloxycarbonyl, t-butoxy-carbonyl, 2-chloro-benzyloxy-carbonyl, 2-chloroethyloxycarbonyl, di-t-amyloxycarbonyl, 9-fluorenyl-methyloxycarbonyl, isopropoxycarbonyl, 4-methoxy-benzyloxycarbonyl,
20 4-nitrobenzyloxycarbonyl, 2-nitrophenyl-sulfonyl, phthaloyl, 2,2,2-trichloro-t-butyloxycarbonyl, trifluoroacetyl, triphenylmethane, allyloxycarbonyl, and vinyloxycarbonyl groups, and the like, in which the preferred ones include benzyloxycarbonyl (carbobenzyloxy), t-butoxy-carbonyl groups, and in which the most preferred one is the t-butoxy-carbonyl group.

In the interest of efficiency, it is preferred that this coupling be conducted *in situ* without isolation of the compound of formula III following its preparation by the aforementioned process. Alternatively,
30 the compound of formula III may be isolated as a discrete intermediate.

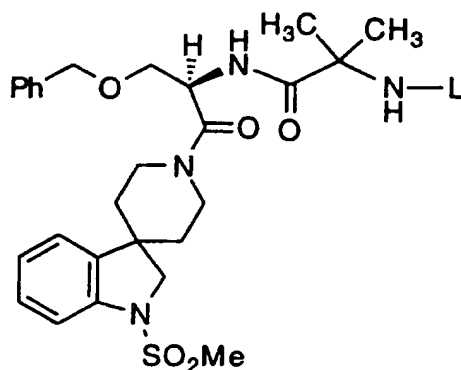
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Within this general process, a fourth process concerns the preparation of a compound of formula IV, or a pharmaceutically acceptable salt thereof:



IV

which comprises reacting a compound of the formula III:



III

wherein L is an amino protecting group, with an amino deprotecting agent to give the compound of formula IV.

Suitable amino protecting groups include: benzyl, benzyloxymethyl, benzyloxycarbonyl (carbobenzyloxy), benzylsulfonyl, 2-bromo-ethyloxycarbonyl, t-butoxy-carbonyl, 2-chloro-benzyloxy-carbonyl, 2-chloroethyloxycarbonyl, di-t-amylloxycarbonyl, 9-fluorenyl-methyloxycarbonyl, isopropoxycarbonyl, 4-methoxy-benzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, 2-nitrophenyl-sulfonyl, phthaloyl, 2,2,2-trichloro-t-butyloxycarbonyl, trifluoroacetyl, triphenylmethane,

- 14 -

allyloxycarbonyl, and vinyloxycarbonyl groups, and the like, in which the preferred ones include benzyloxycarbonyl (carbobenzyloxy), t-butoxycarbonyl groups, and in which the most preferred one is the t-butoxycarbonyl group.

- 5 In this process, the removal of the amino protecting group may be accomplished by use of an appropriate catalytic agent. Removal of a t-butoxycarbonyl protecting group may be carried out in a solvent such as methanol, ethanol, methylene chloride, ethyl acetate, or isopropyl acetate, with a strong acid. Such strong acids include
- 10 methanesulfonic acid, trifluoroacetic acid, hydrochloric acid, hydrogen chloride gas, hydrogen bromide; hydrogen iodide; trifluoromethanesulfonic acid; camphorsulfonic acid; sulfuric acid; phosphoric acid; and an arylsulfonic acid, such as benzenesulfonic acid, p-toluenesulfonic acid, and p-chlorobenzene-sulfonic acid. Preferred catalytic agents include:
- 15 trifluoroacetic acid; methanesulfonic acid; camphorsulfonic acid; benzenesulfonic acid, p-toluenesulfonic acid; and p-chlorobenzene-sulfonic acid. The most preferred catalytic agent is methanesulfonic acid. It is preferred that compound of formula V is isolated in the form of the methanesulfonate salt. The preferred solvent is methanol or ethanol, and
- 20 the most preferred solvent is ethanol.

The preferred reaction temperature range is between -40 and 150°C, and the most preferred range is between 10 and 40°C.

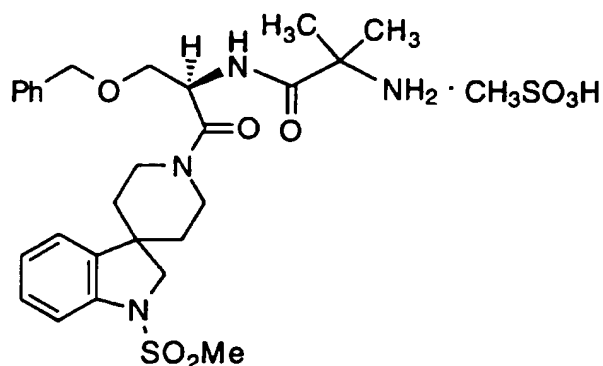
- Removal of a benzyloxycarbonyl (carbobenzyloxy) group may be achieved by a number of methods, for example, catalytic
- 25 hydrogenation with hydrogen in the presence of a noble metal or its oxide such as palladium on activated carbon in a protic solvent such as ethanol. In cases where catalytic hydrogenation is contraindicated by the presence of other potentially reactive functionality, the removal of
- benzyloxycarbonyl (carbobenzyloxy) group may also be achieved by
- 30 treatment with a solution of hydrogen bromide in acetic acid, or by treatment with a mixture of TFA and dimethylsulfide.

In the interest of efficiency, it is preferred that this acid-catalyzed deprotection be conducted *in situ* without isolation of the

- 15 -

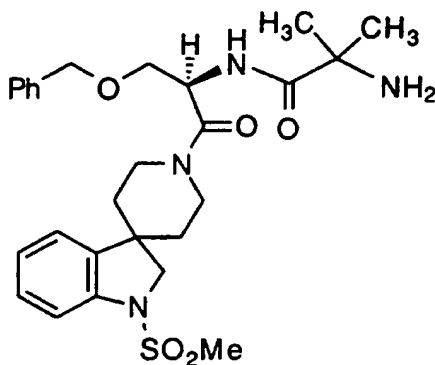
compound of formula IV following its preparation by the aforementioned process.

- 5 Within this general process, a fifth process concerns the preparation of a pharmaceutically acceptable salt of a compound of formula IV, in particular, the methanesulfonate salt, i.e. a compound of formula V:



V

which comprises reacting a compound of the formula IV:



IV

with an acid, preferably methanesulfonic acid, to give the compound of formula V.

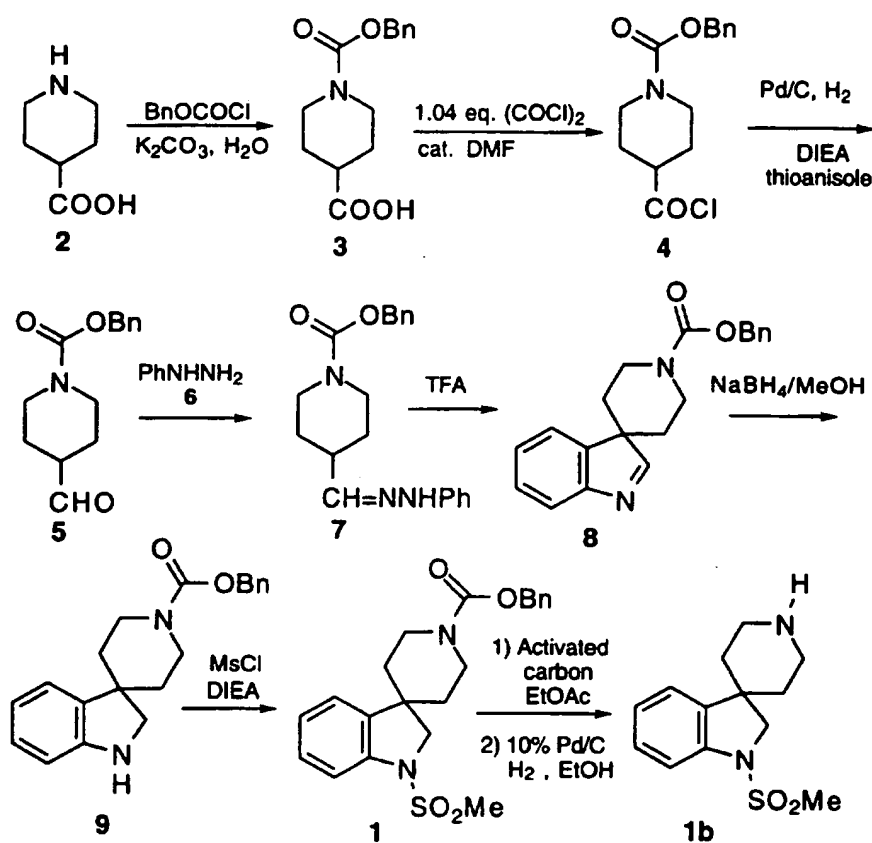
- 15 It is preferred that compound of formula V is isolated in the form of the methanesulfonate salt. The preferred solvent comprises ethyl acetate and ethanol, and the most preferred solvent is a mixture of ethyl acetate and ethanol.

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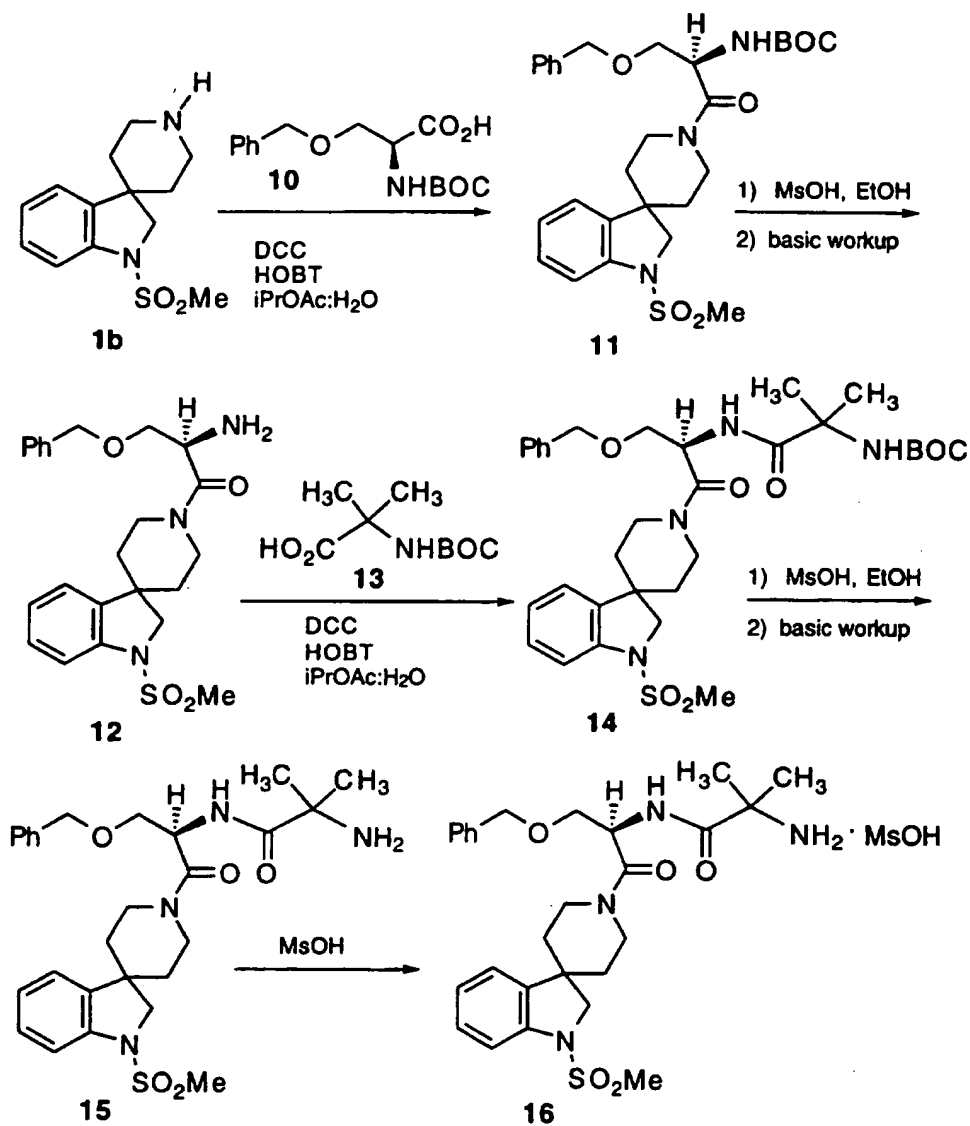
In the interest of efficiency, it is preferred that the formation of the salt be conducted *in situ* without isolation of the compound of formula V following its preparation by the aforementioned process.

- 5 In a preferred embodiment of the present invention, the individual processes within the general process are outlined as follows:

SCHEME II:



- 17 -

SCHEME II (CONT'D)

- 18 -

In this preferred embodiment, the CBZ-Spiroindoline 1 is treated with Darco (20% by weight) prior to hydrogenation. The hydrogenation is carried out in ethanol at 65°C over 10% Pd/C with vigorous stirring.

5 A solution of 1b in isopropyl acetate and water is coupled with commercially available N-BOC-O-benzyl-D-serine in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt). After filtration of the dicyclohexylurea (DCU) side product, the 2-phase filtrate is separated and the organic layer is washed successively with 1M
10 aqueous sodium hydroxide solution, 0.5M aqueous hydrochloric acid and finally saturated aqueous sodium hydrogen carbonate. Improved results in this coupling are achieved when a solution of the free amino in iPrOAc/H₂O is treated with DCC, HOBt followed by addition of the
15 amino acid at ambient temperature and followed by reaction for 3-5 hrs. The batch is then concentrated *in vacuo* and the solvent is switched from isopropyl acetate to ethanol. This solvent switch generally proceeds swiftly by "feeding and bleeding" 3x batch volumes to remove isopropyl acetate..

20 The BOC-group of 11 is removed by treatment with methanesulfonic acid (MsOH) (3 eq) in ethanol at 35-40°C. Partitioning between isopropyl acetate and aqueous 1M sodium hydroxide solution affords 12.

25 The coupling of 12 with N-BOC- α -aminoisobutyric acid is best conducted in a two-phase solvent system, isopropyl acetate/water (1:1) in the presence of DCC and HOBt (1.1 eq. each). Removal of the DCU by filtration, separation of the layers and washing the organic layer successively with 1M aqueous sodium hydroxide, 0.5M aqueous hydrochloric acid and saturated aqueous sodium hydrogen carbonate affords 14.

30 The mixture is solvent switched to ethanol for the subsequent methanesulfonic acid cleavage of the Boc group. Deprotection of 14 is more difficult than that of 11 and requires a concentrated solution of ethanol/methanesulfonic acid and heating to 35-40°C. After extractive workup (EtOAc-NaOH), the free amine 15 is

- 19 -

isolated. The organic layer is washed well with 1N NaOH to ensure complete removal of methanesulfonic acid.

The ethyl acetate solution of the free base 15 is concentrated to low bulk *in vacuo* and is azeotroped dry ($\text{KF} < 500 \text{ mgml}^{-1}$) by
5 "feeding and bleeding" 2x batch volumes of 90/10, ethyl acetate/ethanol followed by 2x batch volumes of ethyl acetate. The resulting dry, slightly hazy solution of the free base 15 in ethyl acetate is treated with Darco G-60 (25 weight %) at room temperature for about 10 hours. Removal of the Darco by filtration with a filtration agent gives the free base 15.

10 Formation of the methanesulfonic acid salt 16 from 15 is carried out in EtOAc with 1.1 eq of MsOH at about 50°C. The free base 15 is treated with 8 volume % of EtOH and 1 eq of H₂O and heated to 55°C until complete dissolution. Cooling to ambient temperature and stirring the resulting slurry for 4 hours gives crystalline material of 16
15 designated as crystal Form II [solubility in IPA = 12 mg/mL].

The conversion of Form II to Form I is accomplished where the salt is formed in EtOAc-EtOH as above, but instead of cooling the initial solution of the salt (at 55°C) to ambient temperature, it is cooled to 45°C. Crystals should start appearing at that temperature and the slurry
20 should become thicker with time. The temperature is then raised to 51°C and the slurry is aged overnight. Complete conversion to Form I of 16 should be expected.

Preferably, the conversion of Form II to Form I is achieved by adding seed crystals of Form I to a solution of the free base in EtOAc-EtOH at 50-55°C followed by aging. Accordingly, the free base 15 may
25 be treated with 1.1 equivs. of methanesulfonic acid in 8% ethanol in ethyl acetate at 50-55°C. The batch is then seeded with approximately 2% by weight of Form I of the methanesulfonate salt 16, and then aged at 55°C overnight. The batch is cooled to room temperature and aged for
30 approximately 2-3 hours. The product is isolated by filtration at room temperature under a nitrogen atmosphere, dried at 35°C *in vacuo* and sieved to give the methanesulfonate salt 16.

The methanesulfonic acid salt 16 may also be formed by alternating the stepwise addition of MsOH (1.1 eq) and seed crystals of

- 20 -

Form I to a solution of the free base in EtOAc-EtOH at about 50°C, wherein the order of addition of the MsOH and the seed is not critical.

Throughout the instant application, the following abbreviations are used with the following meanings:

5	Bu	butyl
	Bn	benzyl
	BOC, Boc	t-butyloxycarbonyl
	BOP	Benzotriazol-1-yloxy tris(dimethylamino)- phosphonium hexafluorophosphate
10	calc.	calculated
	CBZ, Cbz	Benzyloxycarbonyl
	DCC	N,N'-Dicyclohexylcarbodiimide
	DIEA	Di-isopropylethylamine
	DMF	N,N-dimethylformamide
15	DMAP	4-Dimethylaminopyridine
	EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
	EDAC	Ethyl-3-(3-dimethylamino)-propylcarbodiimide
	EI-MS	Electron ion-mass spectroscopy
20	Et	ethyl
	eq.	equivalent(s)
	FAB-MS	Fast atom bombardment-mass spectroscopy
	h, hr.	hours
	HOBT, HOBt	Hydroxybenzotriazole
25	HPLC	High pressure liquid chromatography
	iPrOAc	iso-Propyl acetate
	KHMDS	Potassium bis(trimethylsilyl)amide
	LAH	Lithium aluminum hydride
	LHMDS	Lithium bis(trimethylsilyl)amide
30	Me	methyl
	MF	Molecular formula
	MHz	Megahertz
	MPLC	Medium pressure liquid chromatography
	MsOH	Methane sulfonic acid

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	NMM	N-Methylmorpholine
	NMR	Nuclear Magnetic Resonance
	Ph	phenyl
	Pr	propyl
5	prep.	prepared
	TFA	Trifluoroacetic acid
	THF	Tetrahydrofuran
	TLC	Thin layer chromatography
10	TMS	Tetramethylsilane

In the above structural formula and throughout the instant specification, the following terms have the indicated meanings:

The phrase "peptide coupling reaction" as used herein is intended to mean the coupling of a carboxylic acid with an amine using
15 an acid activating agent such as EDC, DCC, and BOP in an inert solvent in the presence of a catalyst such as HOBT. Inert solvents appropriate for such couplings include: acetonitrile; iso-propyl acetate; ethyl acetate; propionitrile; water; chlorinated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene, ortho-
20 dichlorobenzene; benzene; toluene; xylenes; and combinations thereof; and the like.

The variable "L" and the term "amino protecting group" is intended to indicate the presence of an appropriate protecting group for amino, such as those described in Greene, T.W., Wuts, P.G.M. Protective
25 Groups in Organic Synthesis, 2nd ed., John Wiley & Sons, Inc., New York, 1991. An appropriate protecting group will be able to withstand the reaction conditions of intermediate processes, prior to being removed when desired. The amino protecting group is independently selected for each process within the entire processes.

Suitable amino protecting groups include: benzyl,
30 benzyloxymethyl, benzyloxycarbonyl (carbobenzyloxy), benzylsulfonyl, 2-bromo-ethyloxycarbonyl, t-butoxy-carbonyl, 2-chloro-benzyloxy-carbonyl, 2-chloroethyloxycarbonyl, di-t-amylloxycarbonyl, 9-fluoroenyl-methyloxycarbonyl, isopropoxycarbonyl, 4-methoxy-benzyloxycarbonyl,
35 4-nitrobenzyloxycarbonyl, 2-nitrophenyl-sulfonyl, phthaloyl, 2,2,2-

- 22 -

trichloro-t-butyloxycarbonyl, trifluoroacetyl, triphenylmethane, and vinyloxycarbonyl groups, and the like, in which the preferred ones include benzyloxycarbonyl (carbobenzyloxy), t-butoxy-carbonyl groups, and in which the most preferred one is the t-butoxy-carbonyl group.

5 The removal of the amino protecting group may be accomplished by use of an appropriate catalytic agent. Removal of a t-butoxycarbonyl protecting group may be carried out in a solvent such as methanol, ethanol, methylene chloride, ethyl acetate, or iso-propyl acetate, with a strong acid. Such strong acids include methanesulfonic
10 acid, trifluoroacetic acid, hydrochloric acid, hydrogen chloride gas, hydrogen bromide; hydrogen iodide; trifluoromethane-sulfonic acid; camphorsulfonic acid; sulfuric acid; phosphoric acid; and arylsulfonic acids, such as benzenesulfonic acid, p-toluenesulfonic acid, and p-chlorobenzene-sulfonic acid. Preferred catalytic agents include:
15 trifluoroacetic acid; methanesulfonic acid; camphorsulfonic acid; benzenesulfonic acid, p-toluenesulfonic acid; and p-chlorobenzene-sulfonic acid. The most preferred catalytic agent is methanesulfonic acid. The preferred solvent is methanol or ethanol.

 Removal of a benzyloxycarbonyl (carbobenzyloxy)
20 protecting group may be achieved by a number of methods, for example, catalytic hydrogenation with hydrogen in the presence of a noble metal or its oxide such as palladium on activated carbon in a protic solvent such as ethanol. In cases where catalytic hydrogenation is contraindicated by the presence of other potentially reactive functionality, the removal of
25 benzyloxycarbonyl (carbobenzyloxy) group may also be achieved by treatment with a solution of hydrogen bromide in acetic acid, or by treatment with a mixture of TFA and dimethylsulfide.

 The amine compounds employed as starting materials for the process of the present invention may be present as their acid salts, such as
30 the salts derived from using inorganic and organic acids. Examples of such acids are hydrochloric, nitric, sulfuric, phosphoric, formic, acetic, trifluoroacetic, propionic, maleic, succinic, malonic, methane sulfonic and the like. Similarly the compounds produced by the processes of the instant invention may be isolated in the form of their pharmaceutically

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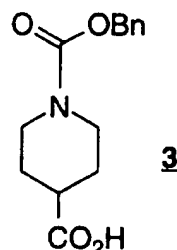
acceptable acid salts. In addition, certain compounds containing an acidic function such as a carboxy can be in the form of their inorganic salt in which the counterion can be selected from sodium, potassium, lithium, calcium, magnesium and the like, as well as from organic bases.

5 The preparation of compounds with the process of the present invention may be carried out in sequential or convergent synthetic routes. It is noted that in some cases the order of carrying out the foregoing reaction schemes may be varied to facilitate the reaction or to avoid unwanted reaction products. In general, the process of the present
10 invention is conducted in a sequential manner as presented herein.

 Many of the starting materials are either commercially available or known in the literature and others can be prepared following literature methods described for analogous compounds. The skills
15 required in carrying out the reaction and purification of the resulting reaction products are known to those in the art. Purification procedures include crystallization, normal phase or reverse phase chromatography.

 The following examples are provided for the purpose of further illustration only and are not intended to be limitations on the disclosed invention.

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EXAMPLE 1Isonipecotic acid-N-benzyl carbamate (3)5 Materials:

Isonipecotic acid (2) T.C.I.	4.02 kg (31.1 mol)
Benzyl chloroformate (Schweitzerhall)	6.91 kg (40.5 mol)
K ₂ CO ₃	10.1 kg (72.9 mol)
Water	40.2 L

10

Isonipecotic acid (2) and K₂CO₃ were dissolved in 40.2 L of water in a 100 L 4 neck flask with mechanical stirring under N₂ and the solution was cooled to 10°C. Benzyl chloroformate was added, maintaining the temperature between 9 and 14°C, and the mixture was warmed up to 22°C after the addition was complete and aged for 58 h. The addition was completed in 4 h at which point the pH was 9.0. After aging for 58 h there was no change in the pH.

The reaction mixture was transferred to a 200 L extractor and washed with 3 × 13 kg (15 L) of IPAC and 1 × 12 L of EtOAc. The aqueous layer was extracted with 8 L of toluene. After the washes the benzyl alcohol content was reduced from 3.8% to 1.4% by HPLC analysis. HPLC analytical: Dupont Zorbax 25 cm RXC8 column with 1.5 mL/min flow and detection at 254 nm; isocratic mixture with 35% MeCN, 65% of 0.1% aqueous H₃PO₄; retention times: 3 = 6.9 min, benzyl alcohol = 3.3 min, toluene = 17.3 min.

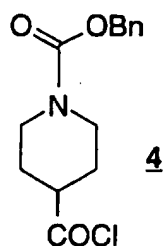
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The aqueous phase was acidified with 37% aqueous HCl to pH 1.8. Carbon dioxide was evolved during the addition of HCl, but gas evolution was easily controlled. The addition of HCl took <1 h and

- 25 -

- required 10 L of conc. HCl. The aqueous phase was extracted with 3 × 6.6 L of toluene. The toluene extracts were dried with 2 kg of sodium sulfate and filtered through a pad of Solka-floc™. The combined filtrates weighed 17.8 kg. The crude yield of carbamate 3 was 7.89 kg (97%) (as obtained by evaporation of weighed aliquots of the filtrates to dryness). The filtrates were transferred through a 10 μ inline filter to a 100 L flask. The extracts were concentrated at 10 mbar at <25°C to a volume of 18 L. The final concentration of carbamate 3 was 440 g/L. The concentration of the toluene filtrate served to azeotropically remove final traces of water (final KF = 170mg/L). The product was 99.1 area % pure with 0.9 area % benzyl alcohol as the only impurity.

EXAMPLE 2



15 Isonipecotic acid chloride-N-benzyl carbamate (4)

Materials:

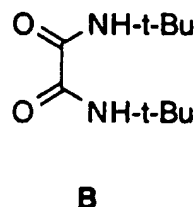
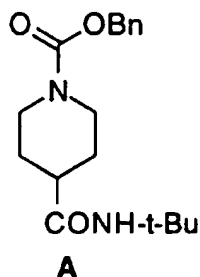
	Isonipecotic acid N-benzyl carbamate (3)	7.89 kg (30.0 mol) in in toluene. (MW = 263.30)	17.9 L
20	Oxalyl chloride (MW = 126.93)	3.94 kg (31.0 mol)	
	DMF (MW = 73.10)	10 mL	
	Toluene	12 L	

- 25 To the toluene solution of benzyl carbamate 3 from the preceding step was added 5 mL of DMF and 10 L of toluene. The oxalyl chloride was added over a period of 20 min. The reaction mixture was aged for 16 h at 18°C under a slow stream of nitrogen. HPLC analysis of the reaction mixture showed that 1.3% of the carboxylic acid 3 still

- 26 -

remained unreacted. The reaction mixture was warmed to 26°C, and 5 mL of DMF were added. The mixture was aged for 2.5 h.

A 1.0 mL aliquot of the reaction mixture was quenched with 5.0 mL of tert-butylamine and analyzed after evaporation by HPLC: 25 cm Dupont Zorbax RXC8 column at 50°C with 1 mL/min flow and detection at 220 nm; isocratic 42% MeCN, 58% of 0.1% aqueous H₃PO₄. This method showed that <0.05% of the acid **3** remained (as judged by **A**) and showed >3 area % **B** (>1 mol% (COCl)₂).



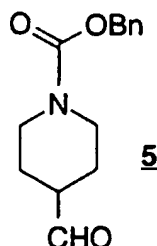
The mixture was concentrated at 10 mbar and a temperature of 20-25°C until 5 L of solvent had been removed.

The typical HPLC profile of concentrated toluene solution after t-BuNH₂ quench described above is as follows:

	<u>Retention time (min)</u>	<u>Area %</u>	<u>Identity</u>
15	2.1	<0.5%	carboxylic acid 3
	7.8	<0.5%	benzyl chloride
	11.0	>99%	Cbz-t-butylcarboxamide A
	12.1	NA	toluene
	12.7	<0.5%	ditert-butylloxamide B

20

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EXAMPLE 3Piperidine-4-carboxaldehyde-1-benzyl carbamate (5)**5 Materials:**

Isonipecotic acid chloride <i>N</i> -benzyl carbamate (4)	3.38 kg (12.0 mol)
in toluene (MW = 281.74)	in 5.54 kg
DIEA (KF = 18 mg/L)	1.55 kg (15.0 mol)
10% Pd/C (KF < 20 mg/g)	101 g
10 thioanisole (MW = 124.21, d = 1.058)	0.56 g

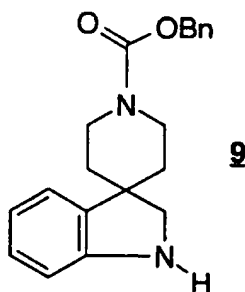
The DIEA and thioanisole were added to the solution of (**4**) in toluene from the previous step and the catalyst was suspended in this mixture. The mixture was immediately placed into the 5 gal autoclave and hydrogenated at 20°C and 40 psi of H₂. After 18 h the reaction had taken up 70% the theoretical amount of hydrogen and HPLC analysis of an aliquot that was quenched with tert-butylamine indicated that 14.2 area % of acid chloride **2** remained. HPLC conditions same as above. Retention time: **5** = 8.1 min.

20 A second charge of catalyst (101 g) and thioanisole (0.54 g) were added as a slurry in 1375 mL toluene to the hydrogenator. After 23 h HPLC analysis of an aliquot that was quenched with tert-butylamine indicated that 1.8 area % of acid chloride **2** remained. The mixture was purged with nitrogen and the catalyst and precipitated DIEA•HCl were removed by filtration through Solka-floc™. The filter cake was washed with 10 L of toluene. The filtrates were transferred through a 10 μ inline filter to a 50 L extractor and washed with 2 × 7.2 L of 1 M aqueous HCl and 2 × 7.2 L of water. The mixture was concentrated at 10 mbar and a temperature of 25-30°C until 5 L of residue remained.

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	<u>Retention time (min)</u>	<u>Area %</u>	<u>Identity</u>
	2.1	<2	carboxylic acid 3
	6.6	<1	dimer 21
5	8.1	>95	aldehyde 5

The assay yield of aldehyde **3** was 94% by HPLC analysis.

EXAMPLE 4

10

CBZ-Spiroindoline (9)Materials:

	Piperidine-4-carboxaldehyde-1-benzyl	1.71 kg (6.89 mol)
15	carbamate (5) in toluene solution	in 21.4 kg
	Phenylhydrazine	900 mL, 981 g (9.15 mol)
	Trifluoroacetic acid (TFA)	2.20 L, 3.26 kg (28.6 mol)
	NaBH ₄	300 g, (7.93 mol)
	Toluene	34.4 kg
20	MeCN	7.0 L
	MeOH	7.0 L

The crude aldehyde **5** solution from the previous step was transferred through a 10 μ inline filter to a 100 L reactor equipped with

25 Teflon coated copper coils for cooling or heating and a mechanical stirrer. Toluene (34.4 kg) and MeCN (7 L) were added, and the resulting solution was cooled to 0°C. Phenylhydrazine was added in portions and the

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temperature was maintained at -1 to 3°C while nitrogen was continuously bubbled through the reaction mixture.

- The phenylhydrazine was added until TLC and HPLC analysis indicated complete consumption of the aldehyde 5 and the appearance of a slight excess (<5%) of phenylhydrazine. TLC conditions: Silica, E. Merck Kieselgel G60 F254 0.25 mm; diethyl ether/pentane (4/1); and developing agent 0.5% ceric sulfate, 14% ammonium molybdate in 10% aqueous sulfuric acid then heat; R_f: aldehyde 5 = 0.52, phenylhydrazone 7 = 0.61, phenylhydrazine 6 = 0.21. HPLC conditions: 25 cm Dupont Zorbax RXC8 column at 30°C with 1.0 mL/min flow and detection at 254 nm; gradient schedule:

	<u>Time (min)</u>	<u>acetonitrile:water</u>
	0	57:43
15	10	65:35
	15	75:25
	18	75:25

retention times: phenylhydrazine 6 = 4.5 min, toluene = 7.2 min, phenylhydrazone 7 = 11.4 min.

- The reaction mixture was aged for 30 min at 0-2°C, and TFA was added maintaining the temperature between 2 and 7°C. The reaction mixture was warmed to 50°C over 30 min, and maintained for 17 h. The nitrogen sparge through the reaction mixture was stopped and a slow stream of nitrogen was maintained over the reaction mixture. During the first hour at 5°C the color gradually darkened to a deep green, and a relatively small amount of a white crystalline precipitate (ammonium trifluoroacetate) formed. After 17 h HPLC analysis (same conditions as above) indicated that the reaction mixture contained 91.6 area % indolenine 8 and 1.5% of unreacted phenylhydrazone remained. Aging the mixture for longer periods of time did not increase the assay yield of indolenine 8.

The reaction mixture was cooled to 12°C, and 7.0 L of MeOH was added. NaBH₄ was added in small (<20 g) portions maintaining the temperature below 15°C. The addition took 30 min.

- 30 -

Moderate hydrogen evolution was observed during the addition, but it was easily controlled and there was virtually no frothing. Near the end of the addition the color rapidly changed from green to brown and then bright orange. A small amount (<200 mL) of a heavier phase had separated (presumably aqueous salts). HPLC analysis (conditions as before) indicated that all of the indolenine 8 had been consumed (90.4 area % CBZ-indoline 9); retention times: indolenine 8 = 7.5 min, indoline 9 = 8.2 min. TLC: ethyl ether as solvent, ceric sulfate-ammonium molybdate stain or 1% anisaldehyde stain; retention factors: indolenine 8 = 0.18, CBZ-indoline 9 = 0.33.

The color change from green to orange corresponds very closely to reaction end point. The quantity of NaBH₄ required to complete the reaction is heavily dependent on the temperature and rate of addition of NaBH₄, but the yield and quality of the product is virtually unaffected provided that the reaction is complete. The reaction mixture was cooled to 5°C over a period of 30 min. Then 8 L of 3% aqueous NH₄OH (8 L) were added to bring the pH of the aqueous phase to 7.4, the mixture was agitated, and allowed to settle. The temperature rose to 15°C. The cloudy yellow lower aqueous phase was separated. The organic phase was washed with 4 L of 3% aqueous NH₄OH, 2 × 4 L of water, and 2 × 4 L of brine. The weight of the organic phase after the washings was 53.5 kg, and the assay yield was 94%.

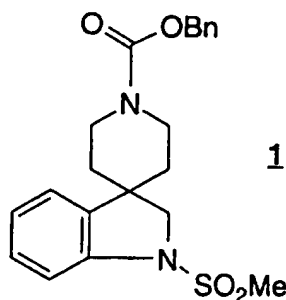
The washed toluene solution was combined with the washed organic phases of two other similarly processed reactions. The total aldehyde used in the three reactions was 5.06 kg, (20.5 mol). The total weight of CBZ-indoline 9 assayed in the combined organic phases was 5.91 kg, (18.3 mol, 90% assay yield). The combined organic phases were dried with 5 kg of sodium sulfate, treated with 250 g of Darco G60 carbon for 30 min, and filtered through Solka-floc™. The filtrates were vacuum concentrated at 10 mbar at <25°C until the residue was near dryness. The solvent switch was completed by slowly bleeding in 30 L of IPAC and reconcentrating to 14 L at 200 mbar at 50-60°C. The mixture was heated to reflux in order to obtain a clear homogeneous deep orange

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solution. ^1H NMR analysis indicated that the solution contained ca. 6 mol% of residual toluene after solvent switch.

The solution was cooled to 68°C and seeded with 4 g of crystalline CBZ-indoline 9. The solution was allowed to gradually cool to 26°C over 6 h and aged for 9 h at 20-26°C. The slurry was cooled to 2°C over 1 h and aged at 2°C for 1h. The product was isolated by filtration, and the filter cake was washed with 2 × 2 L of 5°C IPAC and 2 × 2 L of 5°C MTBE. The product was dried in the vacuum oven at 30°C under a nitrogen bleed to give 4.37 kg (74%) of the title compound 9 as a light tan crystalline powder. HPLC analysis of the product indicated 99.5 area % purity. The mother liquor (11 L) and the washes contained 1.15 kg (19%) of additional product 9 and ca 3% of Cbz-isonipecotic acid phenylhydrazide (retention time = 4.8 min).

15

EXAMPLE 5CBZ-Spiroindoline-methanesulfonamide (1)Materials:

20	CBZ-Spiroindoline (9)	1.69 kg (5.23 mol)
	Methanesulfonyl chloride	599 g (5.23 mol)
	Et ₃ N (KF = 151)	635 g (6.27 mol)
	THF (KF = 41)	12 L

25

A 22 L flask was charged with the solid CBZ-spiroindoline 9 and then 11.5 L of THF and the Et₃N were transferred into the flask through a 10 μ inline filter. The resulting homogenous solution was

- 32 -

cooled to 0°C. A 1 L dropping funnel was charged with the methanesulfonyl chloride and 500 mL of THF. The solution of the MsCl in THF was added to the reaction mixture maintaining the temperature between 0 and 4°C. The addition took 5 h and was exothermic. A white precipitate, presumably triethylammonium hydrochloride formed during the addition. HPLC analysis indicated that the reaction was complete at the end of the addition (9 was undetectable).

HPLC conditions: 25 cm Dupont Zorbax RXC8 column with 1.5 mL/min flow and detection at 254 nm. Gradient Schedule:

10

Time (min) 0.1% aq. H₃PO₄:MeCN

0 70:30

3 70:30

12 20:80

15

25 20:80

Retention times: 9 = 7.6 min, 1 = 13.6 min.

After the addition was complete the reaction mixture was warmed to 18°C and aged for 16 h. There was no change in the appearance of the reaction mixture, and HPLC profile between the end of the addition and after the 16 h age. The reaction mixture was slowly transferred over 1h into a vigorously stirred solution of 30 L of water and 200 mL of 37% aqueous HCl in a 50 L flask. The temperature in the 50 L flask rose from 22 to 28°C. The product separated as a pale tan gummy solid which changed to a granular solid. The aqueous suspension was cooled to 22°C and aged for 1 h. The suspension was filtered, and the filter cake was washed with 2 × 4 L of MeOH/water (50/50). HPLC analysis indicated that <0.1% of the CBZ-Spiroindoline-methanesulfonamide1 was in the mother liquors.

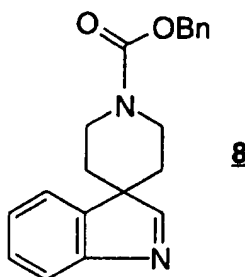
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The filter cake was washed with 4 L of MeOH/water (50/50) to which 50 mL of 28% aqueous NH₄OH had been added. The filter cake was washed with 2 × 4 L of MeOH/water (50/50), and the solid was dried in the vacuum oven at 50°C under a nitrogen bleed to give 2.03 kg

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(97%) of the title product 1 as an off-white powder. HPLC analysis of the solids indicated 93.7 area % 1.

EXAMPLE 6



5

Optional Procedure for Isolation of Intermediate CBZ-Spiroindolenine (8)

Materials:

10	Piperidine-4-carboxaldehyde-1-benzyl carbamate (5)	12.37 g (0.050 mol)
	Phenylhydrazine	5.41 g (0.050 mol)
	Trifluoroacetic acid (TFA)	11.56 mL, 17.10 g (0.150 mol)
15	Methylene chloride	500 mL

The CBZ-aldehyde 5 was dissolved in dichloromethane in a 1 L flask equipped with Teflon coated magnetic stirring bar. The resulting solution was cooled to 0°C. Phenylhydrazine was added via a weighed syringe over 5 min and the temperature was maintained at -1 to 3°C while nitrogen was continuously bubbled through the reaction mixture.

TLC and HPLC analysis indicated complete consumption of the CBZ-aldehyde 5 and the appearance of a slight excess (<2%) of phenylhydrazine. TLC conditions: Silica, E. Merck Kieselgel G60 F254 0.25 mm; diethyl ether/pentane (4/1); and developing agent 0.5% ceric sulfate, 14% ammonium molybdate in 10% aqueous sulfuric acid then heat; R_f: aldehyde 5 = 0.52, phenylhydrazone 7 = 0.61, phenylhydrazine

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6 = 0.21. HPLC conditions: 25 cm Dupont Zorbax RXC8 column at 30°C with 1.0 mL/min flow and detection at 254 nm; gradient schedule:

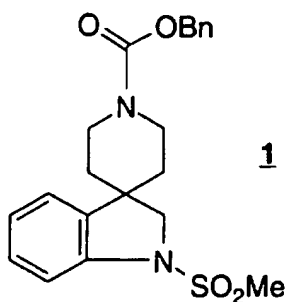
	<u>Time (min)</u>	<u>acetonitrile:water</u>
5	0	57:43
	10	65:35
	15	75:25
	18	75:25

retention times: phenylhydrazine 6 = 4.5 min, toluene = 7.2 min,
10 phenylhydrazone 7 = 11.4 min.

The reaction mixture was aged for 10 min at 0-2°C, and TFA was added by syringe maintaining the temperature between 2 and 7°C. The reaction mixture was warmed to 35°C over 30 min, and maintained
15 for 17 h. The nitrogen sparge through the reaction mixture was stopped and a slow stream of nitrogen was maintained over the reaction mixture. During the first hour at 35°C the color gradually darkened to a rosy pink then to a deep green, and a relatively small amount of a white crystalline precipitate (ammonium trifluoroacetate) formed. After aging for 17 h
20 HPLC analysis (same conditions as above) indicated that the reaction mixture contained 93 area % indolenine 8 and <0.5% of unreacted phenylhydrazone remained. Aging the mixture for longer periods of time did not increase the assay yield of indolenine 8.

The reaction mixture was cooled to 10°C, and a mixture containing 60
25 mL 28-30% ammonium hydroxide, 90 mL water and 150 g crushed ice was added with good stirring. The color of the mixture changed to a salmon color. The organic phase was separated and washed twice with 400 mL water then 100 mL saturated aqueous NaCl. The organic phase was dried over magnesium sulfate and filtered through a plug of 5 g of
30 silica. The filtrate was evaporated to give 15.84 g (99%) of indolenine 8 as a pale orange oil.

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EXAMPLE 7

Procedure for the Preparation of CBZ-Spiroindoline-methanesulfonamide
(1) without Isolation of Intermediate CBZ-Spiroindoline (9)

5

Step 1: CBZ-Spiroindoline (9)

Materials:

	Piperidine-4-carboxaldehyde-1-benzyl	49.5 g (0.20 mol)
10	carbamate (5)	
	Phenylhydrazine (Aldrich)	23.7 g (0.22 mol)
	Trifluoroacetic acid (TFA)	75.4 g (0.66 mol)
	Toluene (KF < 250 mg/L)	654 mL
	MeCN (KF < 250 mg/L)	13.3 mL
15	NaBH ₄	11.3 g, (0.30 mol)
	Toluene	20 mL
	MeOH	50 mL

20 A 2% (by volume) solution of MeCN in toluene was made up using 654 mL of toluene and 13.3 mL of MeCN. In a 2 L 3 neck flask equipped with a mechanical stirrer 617 ml of the above solution were degassed by passing a fine stream of nitrogen through the solution for 5 min. Phenylhydrazine and TFA were added to the mixture while still degassing.

25 The CBZ-aldehyde 5 was dissolved in the rest of the solution prepared above (50 mL) and degassed by bubbling nitrogen through the solution while in the addition funnel. The solution in the flask was heated

- 36 -

to 35°C, and the aldehyde solution was slowly added to the phenylhydrazine-TFA over 2 h. The mixture was aged at 35°C for 16h.

HPLC conditions: 25 cm Dupont Zorbax RXC8 column at 50°C with 1 mL/min flow and detection at 220 nm; isocratic 55% MeCN, 45% of 0.1% aqueous H₃PO₄. Typical HPLC profile after 16 h age:

	<u>Retention time (min)</u>	<u>Area %</u>	<u>Identity</u>
	1.6	0.1-0.5	phenylhydrazine 6
	4.1	<0.1	dimer 21
	4.7	<0.1	aldehyde 5
10	5.0	NA	spiroindoline 9
	6.3	NA	toluene
	6.9	97	spiroindolenine 8
	10.3	<0.2	phenylhydrazone 7
		2-3 tot.	other impurities <0.2% ea.

15

The mixture was cooled to -10°C and MeOH was added. A suspension of sodium borohydride in 20 mL toluene was added in small portions (1 mL) over 30 min taking care that the temperature did not exceed -2°C.

	<u>Area %</u>	<u>Identity</u>
20	0.1-1	phenylhydrazine 6
	85-90	CBZ-spiroindoline 9
	<0.1	CBZ-spiroindolenine 8
	10-15 tot.	other impurities (<3% ea.)

25

The temperature was raised to 10°C over 1h, and 6% aqueous ammonia (200 mL) was added. The mixture was agitated for 10 min, allowed to settle for another 10 min, and the lower aqueous phase was drawn off. Acetonitrile (20 mL) and MeOH (20 mL) were added to the organic phase and it was washed with 150 mL of 15% brine. The organic phase was found to contain a 92% assay yield of CBZ-spiroindoline 9.

30 Step 2: CBZ-Spiroindoline-methanesulfonamide (1)

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Materials:

	CBZ-Spiroindoline (9) (MW = 322.51)	(0.184 mol)
	Methanesulfonyl chloride	21.1 g (0.184 mol)
5	DIEA (KF = 150 mg/L)	29.7 g, 40.1 mL (0.230 mol)
	THF (KF = 41 mg/L)	150 mL

The crude solution of CBZ-spiroindoline 9 solution from Step 1 above was concentrated in a 1L 3 neck flask (60-70°C, 150-200 Torr) until 250 g of residue remained. The THF and DIEA were added, and the resulting homogenous solution was cooled to 0°C. A 125 mL dropping funnel was charged with the methanesulfonyl chloride and 50 mL of THF. The solution of MsCl in THF was added over 2 h to the reaction mixture maintaining the temperature between 0 and 4°C and the mixture was aged for 2 h at 5-8°C. The addition was slightly exothermic. A white precipitate, presumably DIEA-hydrochloride, formed during the addition. HPLC conditions were the same as above. HPLC analysis indicated that the reaction was complete 1 h after the end of the addition (9 was undetectable) and the assay yield was 94% from 9. Retention time: 1 = 7.8 min. Typical HPLC profile of reaction mixture after 2 h age:

	<u>Area %</u>	<u>Identity</u>
	<0.1	CBZ-spiroindoline 9
	90-92	CBZ-sulfonamide 1
25	8-10 tot.	other impurities (<2% ea.)

The mixture was warmed to 20°C, and 200 mL of 1M aqueous HCl was added. The mixture was warmed to 50°C, and the aqueous phase was separated. The organic phase was washed sequentially with 100 mL water, 100 mL 5% aqueous sodium bicarbonate, and 100 mL water. The organic phase was transferred to a 1 L 3 neck flask equipped for mechanical stirring and distillation. The mixture (ca 400 mL) was distilled at atmospheric pressure until 150 mL of distillate had been collected. The head temperature reached 107°C; the pot

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temperature was 110°C. The distillation was continued with continuous addition of n-propanol at such a rate as to maintain a constant volume (ca 350 mL) in the pot. The distillation was stopped when a total of 525 mL of n-PrOH had been added and a total of 800 mL of distillate had been collected.

The temperature of both the head and pot rose from 94°C to 98°C during the solvent switch. Toluene and n-PrOH form an azeotrope boiling at 97.2°C composed of 47.5% toluene and 52.5% n-PrOH. The mixture was allowed to cool gradually to 20°C over 3h and aged for 12 h. The mother liquor was found to contain 2% toluene and 4 mg/mL of sulfonamide. The solubility of the sulfonamide in various mixtures of toluene and n-PrOH has been determined by HPLC assay:

	<u>%toluene in n-PrOH</u>	<u>solubility of 1 in mg/mL</u>
15	0	2.36
	5	3.02
	10	4.23
	20	7.51
	25	10.3

20

The crystalline slurry was filtered and washed with 3 x 100 mL of n-PrOH. The product was dried in a vacuum oven at 50°C with a nitrogen bleed for 16 h to furnish 65.5g (82 % from aldehyde 5) of 6 as a tan solid with 93.5 wt% purity.

25

Typical HPLC profile of solid:

<u>Area %</u>	<u>Identity</u>
<0.1	CBZ-spiroindoline 9
>99	CBZ-sulfonamide 1
<1 tot.	other impurities (<0.2% ea.)

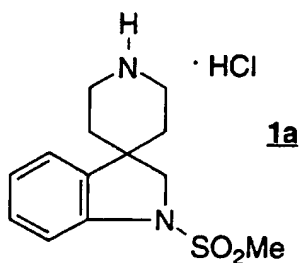
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For additional purification, a 40.0 g sample of the n-PrOH crystallized sulfonamide was dissolved in 134 mL of EtOAc at 60°C and treated with 8.0 g of Darco G-60 carbon for 1 h at 60°C. After the addition of 2.0 g Solkafloc™, the slurry was filtered through a pad of 4.0

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g Solkafloc™, and the pad was washed with 90 mL of EtOAc at 60°C. Prior to the addition of the carbon the solution was a brown color. The filtration proceeded well without plugging to give a golden yellow filtrate. The filtrate was distilled at atmospheric pressure in a 500 mL flask (pot temperature 80-85°C) until 100 g (100 mL) of residue remained. This solution was allowed to cool to 35°C over 3 h. Over a 1 h period, 116 mL of cyclohexane was added with good agitation at 35°C. The mixture was cooled to 20°C over 1 h and aged at 20°C for 12 h. At 35°C much of the sulfonamide has crystallized out and the mixture was thick. Addition of cyclohexane at 20°C makes agitation difficult. After the aging period, the supernatant was found to contain 2.5 mg 1/g. The crystalline slurry was filtered and the cake was washed with 77 mL of 2:1 cyclohexane-EtOAc and 2 x 77 mL of cyclohexane. The product was dried in a vacuum oven at 50°C with a nitrogen bleed for 16 h to furnish 34.2 g of 1 (MW = 400.3) as a white crystalline solid (85 % recovery from crude 1, 70 % from 5 with >99.9 wt % purity).

EXAMPLE 8



20 HCl Salt of Spiroindoline-methanesulfonamide (1a)

Materials:

	CBZ-spiroindoline-methanesulfonamide (1)	941 g (2.35 mol)
	Pearlman's catalyst 20% Pd(OH) ₂ /C	188 g
25	THF	8 L
	MeOH	7 L

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The catalyst was suspended in 7 L of MeOH and transferred into the 5 gal autoclave followed by the solution of 1 in 8 L of THF. The mixture was hydrogenolyzed at 25°C at 80 psi of H₂. After 2.5 h the temperature was raised to 35°C over 30 min.

- 5 HPLC analysis indicated complete consumption of Cbz-spiroindoline-methanesulfonamide. HPLC conditions: 25 cm Dupont Zorbax RXC8 column with 1.5 mL/min flow and detection at 254 nm. Gradient Schedule:

	<u>Time (min)</u>	<u>0.1% aq. H₃PO₄:MeCN</u>
10	0	70:30
	3	70:30
	12	20:80
	25	20:80
	retention times: Spiroindoline = 7.6 min,	
15	Cbz-spiroindoline-methanesulfonamide = 13.6 min.	

- The mixture was purged with nitrogen and the catalyst was removed by filtration through Solka-floc™ while still warm. The catalyst was washed with 4 L of THF and 2 L of MeOH. The pale yellow filtrates were concentrated to a thick oil at 10 mbar and <25°C. The solvent switch was completed by slowly bleeding in 15 L of EtOAc and reconcentrating to dryness. The residue solidified to a hard off-white mass. MeOH (1.5 L) was added and the mixture was heated to 70°C to give a homogenous solution. While the solution was at 70°C, 10.5 L of EtOAc at 20°C was added. The temperature fell to 40°C, and the mixture remained homogenous.

- Subsequent experiments suggested that it is more convenient to solvent switch the MeOH-THF filtrates to MeOH, concentrate to the desired volume, and then add the EtOAc. This avoids the solidification of the residue upon concentration of the EtOAc solution.

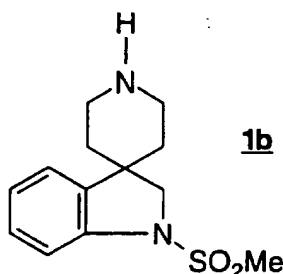
Hydrogen chloride diluted with about an equal volume of nitrogen was passed into the solution. The temperature rose to 60°C over the course of 15 min, and a white precipitate of the hydrochloride salt

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formed. Diluting the HCl with nitrogen only avoids the reaction mixture sucking back and may not be necessary.

- The mixture was cooled in an ice bath, and the hydrogen chloride addition was continued for 1h. The temperature gradually fell to 20°C. The suspension was aged for 2 h while the temperature was lowered to 10°C. The crystalline product was isolated by filtration, and the filter cake was washed with 3 L of EtOAc. It was dried in the vacuum oven at 35°C to give 1.18 kg (86%) of the title product **1a** as an off-white crystalline solid of >99.5 area % purity by HPLC analysis.
- HPLC conditions: 25 cm Dupont Zorbax RXC8 column with 1.5 mL/min flow and detection at 230 nm; isocratic 35% MeCN, 65% of 0.1% aqueous ammonium acetate. Retention time: **1a** = 5.4 min.

EXAMPLE 9



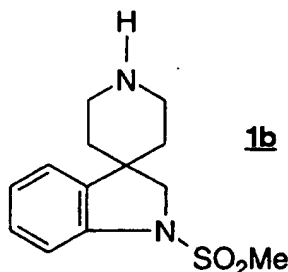
Spiroindoline-methanesulfonamide (Free base form) (**1b**)

- A 250 mL aliquot of the filtrate from the Cbz-hydrogenolysis containing 4.67 g of **1b** (free base) was concentrated to ca 10 mL. The residue was dissolved in 20 mL of EtOAc and the solution was reconcentrated to ca 10 mL. This was repeated once more, and 10 mL of EtOAc was added to the residue. A crystalline precipitate began to form. MTBE (20 mL) was added in one portion. Additional crystalline solid precipitated, but the supernatant still contained a substantial quantity of dissolved product which did not precipitate on standing. Hexanes (70 mL) were added dropwise over 2 h to the mixture with vigorous stirring. The slow addition of the hexanes is necessary to avoid the oiling out of the amine.

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The agitated mixture was aged for 1h and filtered. The filter cake was washed with 20 mL of 1:1 MTBE-hexanes and then with 20 mL of hexanes. The product was dried under a stream of nitrogen to give 3.86 g (82%) of the free amine of **1b** as an off white crystalline solid of >99.5 area % purity. HPLC conditions: 25 cm Dupont Zorbax RXC8 column with 1.5 mL/min flow and detection at 230 nm; isocratic 35% MeCN, 65% of 0.1% aqueous ammonium acetate. Retention time: **1b** = 5.4 min.

10

EXAMPLE 10ASpiroindoline-methanesulfonamide (Free base form) (**1b**)15 Materials:

CBZ-Spiroindoline-sulfonamide (1)	833.5 gr (2.08 mol)
Pd(OH) ₂ /C (20% weight of Pd(OH) ₂)	124.5 (15%)
THF	6.5 L
MeOH	19.5 L
20 NH ₄ OH (conc)	60 mL

The hydrogenation was run three (3) times due to equipment limitations; this procedure refers to a single run. The CBZ spiroindoline sulfonamide **1** was dissolved in THF (6.5 L, KF = 53 µg/µL) and then MeOH (KF=18 µg/mL, 4L) was added followed by addition of the catalyst and the slurry was transferred to a 5 gal autoclave. The remainder of the MeOH (2.5 L) was used for rinsing. The mixture was heated to 40°C at 50 psi for 24 hours. The catalyst loading and reaction

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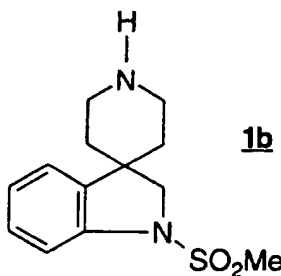
time are a function of the purity of starting material **1**. This material was unique requiring $\geq 15\%$ catalyst and long reaction time. Purer batches of spiroindoline required only 5% of catalyst and 4-6 hrs reaction time.

Upon completion (<0.1 A% **1** by LC) the mixture was
5 filtered thru Solka Floc™ and the carbon cake washed with MeOH (13 L) containing NH₄OH (0.5%, 60 mL). The combined filtrates (assay shows 1587 g of spiroindoline amine **1b**) were concentrated in vacuo and the resulting solids were partitioned between 40 L (of toluene:THF (3:1) and 0.5N NaOH (18 L). Although the layers separated easily a heavy
10 precipitate could be seen in the aqueous layer. The aqueous suspension was thus extracted with CH₂Cl₂ (15 L). The aqueous and organic layer separated slowly. Prior to CH₂Cl₂ addition THF was added to the aqueous layer along with enough NaCl to saturate the layer. However dissolution of the product was not achieved which necessitated the use of
15 CH₂Cl₂.

The combined toluene, THF and CH₂Cl₂ layers were combined and concentrated in the batch concentrator. The residue was flushed with 7 L of CH₃CN. Finally 10 L of CH₃CN were added and the solution stood overnight under N₂ atmosphere.

20

EXAMPLE 10B



Spiroindoline-methanesulfonamide (Free base form) (**1b**)

25 Materials:

CBZ-Spiroindoline-sulfonamide (1)	3 kg (7.49 mol)
Darco G-60	600 g
Ethyl Acetate	36 L

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	Absolute Ethanol	189 L
	10% Pd/C	450 g
	Ammonia Solution	500 ml
	Solka Floc™	2.5 kg
5	Isopropyl Acetate	65 L

A mixture of CBZ-spiroindoline (1) (1 kg) and Darco G-60 (200 g) in ethyl acetate (9 L) was stirred and heated at 60-65°C under a nitrogen atmosphere for 8 hours. The Darco was removed by filtration at 60-65°C, the solid washed with hot ethyl acetate (3 L) and the filtrate and washings combined. LC wt/wt assay confirmed negligible loss to the Darco. The ethyl acetate solution was evaporated to dryness *in vacuo* using a 20 L Buchi apparatus and then flushed with absolute ethanol (2 x 5 L). This material was then slurried in absolute ethanol (8 L) warmed to 65-70°C and placed in the 20 L autoclave. The batch was rinsed in with absolute ethanol (1 L). A slurry of 10% Palladium on charcoal (75 g, 7.5% by weight) in absolute ethanol (750 ml) was then added to the autoclave and rinsed in with a further portion of absolute ethanol (250 ml).

The batch was hydrogenated at 65°C with vigorous stirring under 40 psi hydrogen pressure for 3 hours, a second portion of 10% palladium on charcoal (75 g) was added, the batch was hydrogenated for a further 2 hours and then sealed overnight. The batch was transferred (still hot, 60-65°C) to a 20 L Buchi apparatus and degassed *in vacuo* to remove formic acid by "feeding and bleeding" absolute ethanol (18 L total).

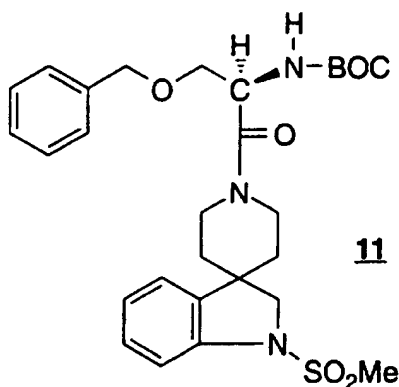
This procedure was repeated twice more and the three batches were combined in a 10 gallon glass-lined vessel and the combined batch was degassed again by the addition and distillation (*in vacuo*) of absolute ethanol (2 x 10 L). Solka floc™ (0.5 kg) was added to the batch and rinsed in with ethanol (10 L). An Estrella filter was loaded with Solkafloc™ (2 kg) as a slurry in ethanol (20 L). The resulting mixture was warmed to 60-65°C and then transferred at this temperature *via* heated filter using pump to two tared stainless-steel bins. The initial

- 45 -

vessel, the filter, the pump and the lines were rinsed with a hot (60-65°C) mixture of aqueous ammonia (500 ml) in absolute ethanol (25 L). The filtrate and washings were combined in the two stainless-steel bins.

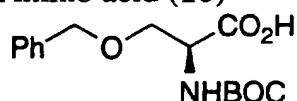
The batch was then transferred to a vessel using an in-line
5 filter containing a 10 micron cartridge, and then concentrated *in vacuo* to low bulk (~15 L). The ethanol was replaced by isopropyl acetate by the "feeding and bleeding" of 3x batch volumes of isopropyl acetate (45 L total), while maintaining a batch volume of ~15 L. The solvent switch, when complete, contained <1% residual ethanol by GC. The batch was
10 then diluted to ~33 L by the addition of isopropyl acetate (20 L), and this solution of spiroindoline-amine 1b (1.855 kg by LC analysis) in isopropyl acetate was used for the next stage of the process.

EXAMPLE 11A



Boc-O-Benzylserine Spiroindoline (11)

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Materials:Spiroindoline-amine (**1b**) 1587 g (5.966 moles)Amino acid (**10**) 1938 g (6.563 moles)

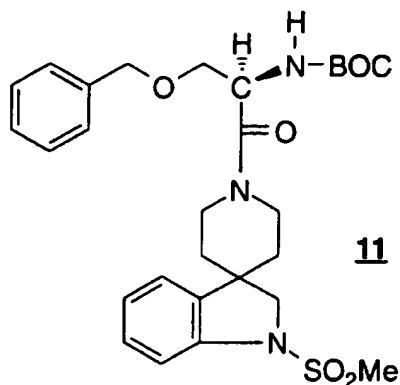
5	DCC	1334.5 g (6.563 moles)
	HOBT	884 g (6.563 moles)
	CH ₃ CN	25 L
	0.5N NaOH	18 L
	0.5N HCl	18 L
10	NaHCO ₃ sat.	18 L
	iPrOAc	28 L

The spiroindoline-amine **1b** in CH₃CN or iPrOAc:H₂O (25 L) at ambient temperature under N₂ was treated in sequence with HOBT (884 g; 1.1 eq) as a solid, DCC (1334.5 g, 1.1 eq) as the melt (heating in hot water at 60°C for ca. 1 hr) and finally the amino acid **10** (1938 g) as the solid. The mixture was stirred for 3 hr upon which time heavy precipitation of DCU occurred and LC analysis showed ca. 0.5 A% of amine **1b** remaining. IPAc (9 L) was added, the slurry was filtered through Solka Floc™ and the cake was washed with IPAc (19 L). The combined organic solution was washed in sequence with 0.5N NaOH (18 L), 0.5N HCl (18 L) and saturated NaHCO₃ (18 L). A final water wash at this point resulted in an emulsion and was thus eliminated.

The organic layer was concentrated in vacuo and the residue was dissolved in MeOH or EtOH (10 L final volume). Assay yield 3026 gr (89%).

The use of alternative peptide coupling agents such as carbonyldiimidazole or formation of mixed anhydrides, such as sec-butyl carbonate, gave inferior yields of **11** and/or **14** with a high degree of epimerization in the case of the former compound. Other peptide coupling reagents were prohibitively expensive.

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EXAMPLE 11BBoc-O-Benzylserine Spiroindoline (11)5 Materials:

	Spiroindoline-amine (1b)	1.855 kg (6.96 mol)
	Isopropyl acetate	29 L
	Dicyclohexylcarbodiimide (DCC)	1.58 kg (7.65 mol)
	1-Hydroxybenzotriazole (HOBt)	1.03 kg (7.62 mol)
10	N-Boc-O-benzyl-D-Serine	2.26 kg (7.65 mol)
	1M Aqueous sodium hydroxide	26 L
	0.5M Aqueous hydrochloric acid	26 L
	Satd. Aqueous sodium hydrogen carbonate	26 L
	Absolute Ethanol	50 L

15

Water (20 L) was added to a stirred solution of the spiroindoline-amine 1b (1.855 kg) in isopropyl acetate (33 L) in a reaction vessel. The following chemicals were then added sequentially at room temperature under a nitrogen atmosphere: DCC (1.58 kg, 1.1 equivs.), HOBt (1.03 kg, 1.1 equivs.) and finally N-Boc-O-benzyl-D-Serine (2.26 kg, 1.1 equivs.). The reagents were rinsed in with isopropyl acetate (7 L). The batch was stirred at room temperature under nitrogen atmosphere for 5 hours when LC showed the ratio of product/starting material to be 99.4/0.6. The mixture was then filtered through an Estrella filter using cloth and cardboard only and utilizing a pump into another vessel. The

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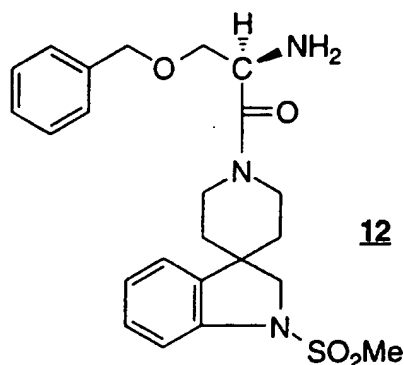
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5 sending vessel was rinsed with isopropyl acetate (22 L) and this was used to rinse the filter, the pump and the lines into the receiving vessel. The 2-phase mixture in the vessel was stirred for 10 minutes and then allowed to settle for 15 minutes. The lower aqueous layer was separated off and the organic solution was left to stand at room temperature overnight.

10 The next day, the organic solution was washed with 1M aqueous sodium hydroxide solution (26 L) then 0.5M aqueous hydrochloric acid (26 L) and finally saturated aqueous sodium hydrogen carbonate (26 L). LC analysis gave an assay yield of 3.787 kg, 93% overall yield from 7.49 moles (3 kg) of starting CBZ-spiroindoline (1). The batch was concentrated *in vacuo* (internal temperature = 13-15°C, jacket temperature = 40°C, Vacuum = 29") to low bulk (~15 L) and solvent switched to ethanol by "feeding and bleeding" ethanol (50 L) whilst maintaining the volume at ~15 L. GC showed <1% isopropyl acetate remaining. This solution was used for the next stage of the process.

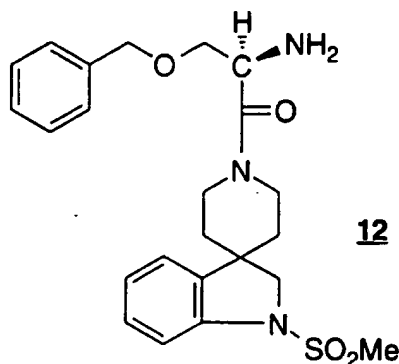
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EXAMPLE 12AO-Benzylserine Spiroindoline (free base form) (12)

5	<u>Materials:</u>	
	Boc-O-Benzylserine Spiroindoline (11)	3026 g (5.57 moles)
	Methane sulfonic acid (MsOH)	1.16 L (17.9 moles)
	MeOH	10 L
	iPrOAc	24 L
10	0.5 N NaOH	35 L

The Boc-O-benzylserine spiroindoline **11** in 10 L of MeOH (or EtOH) was treated with neat MsOH (1.16 L) added over ca. 30-40 min, (initial temperature 16°C, final temperature 28°C). The dark red solution was aged overnight under N₂. The mixture was then pumped into a 100 L extractor containing 24 L iPrOAc and 35 L 0.5 N NaOH. The pH of the aqueous layer was 7. NaOH (6M) was added until pH ≥ 10.5. As the pH increased the color changed from red to yellow. The layers were separated and the organic layer (24 L) was shown by NMR to contain 13 mole % of MeOH in iPrOAc [5 volume %]. LC assay 2.48 kg.

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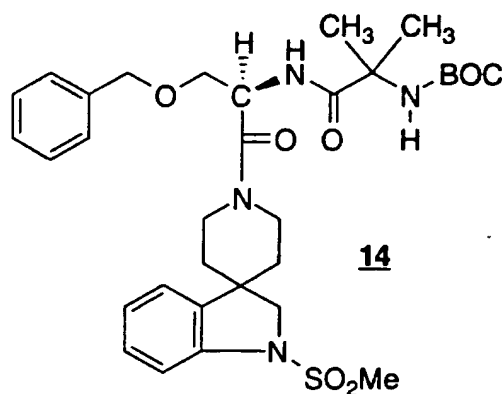
EXAMPLE 12BO-Benzylserine Spiroindoline (free base form) (12)

- 5 Materials:
- | | |
|---------------------------------------|---------------------|
| Boc-O-Benzylserine Spiroindoline (11) | 3.787 kg (6.96 mol) |
| Methanesulphonic acid | 2.006 kg (20.87mol) |
| Isopropyl acetate | 38 L |
| 1M Aqueous sodium hydroxide | 16 L |
| 10 50% Aqueous sodium hydroxide | 1.6 L |

Methanesulphonic acid (2.006 kg, 1.355 L, ~3 equivs.) was added to the stirred solution of Boc-O-benzylserine spiroindoline (11) (3.787 kg) in ethanol (total volume ~15 L) in a reaction vessel. The batch was warmed to 35-40°C. After 7 hours, LC showed the absence of starting material and the reaction was allowed to cool to room temperature overnight. The next day, water (44 L) was added to the batch with stirring. The batch was cooled to ~5°, stirred for 30 minutes and then filtered through an in-line filter (loaded with a 10µ cartridge) into a bin. The batch was then sucked back into the vessel. A water rinse (10 L) was used to rinse the vessel and lines into the bin and this was used to then rinse back into the vessel. Isopropyl acetate (38 L) was added followed by a 1M aqueous sodium hydroxide (16 L). The batch was cooled to 10-15°C, the pH of the lower aqueous layer was confirmed as ~7 and 50% aqueous sodium hydroxide solution was added (1.6 L) (pH >10). The batch was stirred at 10-15°C for 25 minutes and then allowed

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to settle for 10-15 minutes. The lower aqueous layer was separated (78.1 kg). LC assay indicated 28.4 g of 12 (0.85% of theory) contained in the aqueous liquors. Volume of the organic solution = 51 L. LC assay indicated 3.057 kg, 92% overall yield from 3 kg, 7.49 moles of CBZ-spiroindoline sulfonamide (1). This solution was used for the next stage.

EXAMPLE 13A**Boc-Aminoisobutryl O-Benzylserine Spiroindoline (14)**

10

Materials:

	Spiroindoline amine (12)	2481 g (5.57 moles)
	amino acid peptide (13)	1247.1 g (6.16 moles)
	$\begin{array}{c} \text{CH}_3 \quad \text{CH}_3 \\ \diagdown \quad \diagup \\ \text{HO}_2\text{C} \quad \text{NH-BOC} \end{array}$	
15	DCC	1266.7 g (6.16 moles)
	HOBT	827 g (6.16 moles)
	IPAc	52 L
	H ₂ O	37 L
	0.5N NaOH	36 L
20	0.5N HCl	36 L
	Sat. NaHCO ₃	36 L

The solution of the amine 12 in IPAc was diluted to a total volume of 39 L with IPAc and 37 L of H₂O was added. The biphasic

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mixture was then treated in sequence with HOBt (827 g) as a solid, DCC (1266.7 g) as a melt, and amino acid 13 at ambient temperature under nitrogen. The reaction mixture was stirred for 2 h upon which time LC analysis indicated disappearance of the starting material 12 (<0.3 A%).

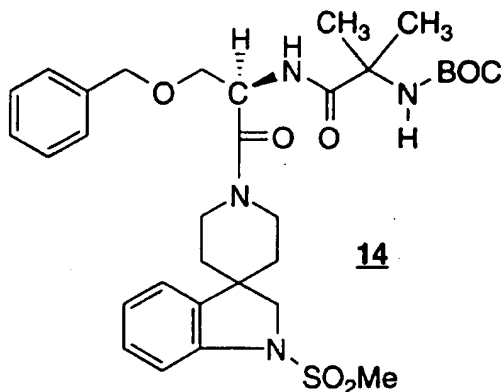
- 5 The mixture was filtered through Solka Floc™ and the solids were washed with 13 L of IPAc. The material may be stored at this point as a biphasic mixture overnight.

- The mixture was transferred to a 100 L extractor, the aqueous layer was separated and the organic layer was washed
10 successively with 36 L of 0.5N NaOH, 0.5N HCl and saturated NaHCO₃. Assay yield 3160 g (81% from spiroindoline ± 5% for volume measurement error). The solution was concentrated to a small volume and was flushed with ethanol (2 x 4 L). If desired, the intermediate compound 14 may be isolated by adding water to crystalize it out.

- 15 The use of alternative peptide coupling agents such as carbonyldiimidazole or formation of mixed anhydrides, such as sec-butyl carbonate, gave inferior yields of 14 with a high degree of epimerization. Other peptide coupling reagents were prohibitively expensive.

20

EXAMPLE 13B



Boc-Aminoisobutyryl O-Benzylserine Spiroindoline (14)

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Materials:

	Spiroindoline amine (12)	3.057 kg (6.89 mol)
	Dicyclohexylcarbodiimide (DCC)	1.56 kg (7.56 mol)
	1-Hydroxybenzotriazole (HOBt)	1.02 kg (7.55 mol)
5	Boc-2-Aminoisobutyric acid (13)	1.54 kg (7.58 mol)
	Isopropyl acetate	32 L
	1M Aqueous sodium hydroxide	38 L
	0.5M Aqueous hydrochloric acid	38 L
	Satd. aqueous sodium hydrogen carbonate	38 L
10	Absolute ethanol	45 L

- Water (49 L) was added to the stirred solution of the spiroindoline amine 12 (3.057 kg) in isopropyl acetate (total volume ~51 L) in a reaction vessel at room temperature under a nitrogen atmosphere.
- 15 The following chemicals were then added sequentially: DCC (1.56 kg, ~1.1 equivs.), HOBt (1.02 kg, ~1.1 equivs.) and finally, N-Boc-2-aminoisobutyric acid 13 (1.54 kg, ~1.1 equivs.). The mixture was stirred vigorously at room temperature for 2 hours when LC showed the reaction to be complete. The mixture was filtered to to another vessel *via* an
- 20 Estrella filter using a pump. Isopropyl acetate (22 L) was used to rinse vessel, the filter, the pump and the lines into the receiving vessel. The 2-phase mixture was then stirred for 5 minutes and the layers were allowed to separate. The lower aqueous layer was separated without incident (weight of aqueous liquors = 51.1 kg). The organic solution was then
- 25 washed sequentially with 1M aqueous sodium hydroxide (38 L), 0.5M aqueous hydrochloric acid (38 L) and finally, saturated aqueous sodium hydrogen carbonate (38 L) without incident.

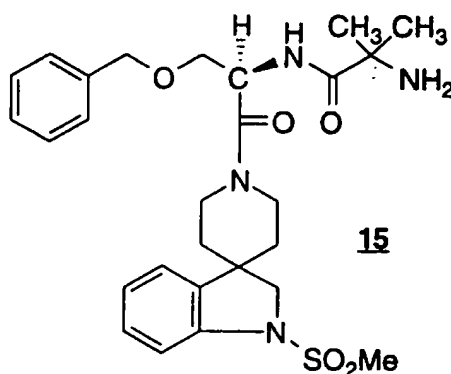
- The organic solution was then transferred using a pump *via* an in-line filter (containing a 10 μ cartridge) to another vessel for the
- 30 solvent switch to ethanol. The vessel was rinsed with isopropyl acetate (10 L) and this was used to rinse the pump, the filter and the lines into the receiving vessel. The filtrate and washings were combined. Total volume = 75 L (by dipstick). LC assay gave 4.395 kg of Boc-

- 54 -

aminoisobutyryl O-benzylserine spiroindoline (**14**), i.e. 93% overall from 7.49 moles of starting CBZ-spiroindoline sulfonamide (**1**).

The batch was concentrated *in vacuo* to low bulk (~15 L) and the isopropyl acetate switched to ethanol by "feeding and bleeding" absolute ethanol (45 L total). At the end of the solvent switch, GC showed <1% isopropyl acetate remaining. This solution (25 L) containing 4.395 kg of **14** was used for the next stage. If desired, the intermediate compound **14** may be isolated by adding water to crystallize it out.

10

EXAMPLE 14AAminoisobutyryl O-Benzylserine Spiroindoline (**15**)15 Materials:

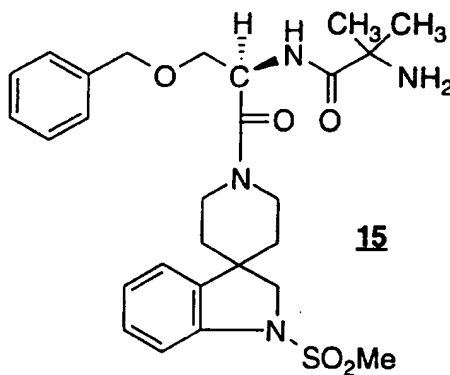
Boc Spiroindoline (14)	3160 g (5.03 moles)
Methanesulfonic acid (MsOH)	979 mL (15.1 moles)
EtOH	6.2 L
H ₂ O	30 L
20 1N NaOH	11 L
EtOAc	26 L
Darco 60 activated carbon	1 Kg

The Boc spiroindoline **14** was dissolved in 6.2 L of EtOH and treated with MsOH (979 mL). The temperature rose from 20 to 30°C and the reaction was allowed to proceed overnight. After 12 hours at

25

- 55 -

- 20°C there was still 15 A% of starting material left so the mixture was heated to 35°C for 6 hours. Upon completion (<0.1 A% 14) the reaction was cooled to 20°C and 30 L of H₂O were added and the solution was filtered through a glass funnel with a polypropylene filter to filter off residual DCU. The mixture was transferred to a 100 L extractor and 26 L of EtOAc were added. The aqueous layer was basified via addition of chilled 1N NaOH (11 L) and 1 L of 50% NaOH. Addition of ice was required to keep the temperature below 14°C. Higher temperatures resulted in significant emulsion problems.
- 10 The organic layer was distilled at 50°C at ca. 21" of Hg until KF <1000 µg/mL. Lower KF's result in more efficient carbon treatments and better recovery at the salt formation step. KF's of 160 µg/mL were achieved at the 700 g scale. The solution was diluted with ethyl acetate to a total volume of 31 L (LC assay 2.40 kg). Activated carbon (Darco
- 15 G-60) was added and the mixture was stirred for 24 h. The mixture was filtered through Solka Floc™ and the filter cake was washed with ethyl acetate (16 L), assay 2.34 Kg.

EXAMPLE 14B

Aminoisobutyryl O-Benzylserine Spiroindoline (15)

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Materials:

	Boc Spiroindoline (14)	4.395 kg (6.99 mol)
	Methanesulfonic acid	2.017 kg (20.99 mol)
	Ethyl acetate	185 L
5	1M Aqueous sodium hydroxide	16 L
	50% Aqueous sodium hydroxide	2.6 L
	Darco G-60	900 g
	Solka Floc™	2.5 kg

- 10 Methanesulfonic acid (2.017 kg, 1.36 L, ~3 equivs.) was added to the stirred solution of the Boc spiroindoline 14 (4.395 kg) in ethanol (total volume ~25 L) in a reaction vessel at room temperature. The batch was warmed to 35-40°C, and stirred overnight. On the next day, the batch contained ~1.1 A% of starting material and so the reaction
- 15 was continued for a further 4 hours, then LC showed ratio of product/starting material to be 99.6/0.4. The batch was concentrated *in vacuo* to ~15 L volume and then diluted with water (44 L). The batch was cooled to 5°C, stirred for 30 minutes and then filtered through a Sparkler in-line filter (containing a 10µ cartridge) using a pump to another vessel to
- 20 remove a small amount of residual DCU.

- The vessel, the pump, the filter and the lines were rinsed with water (10 L), and this was added to the vessel. Ethyl acetate (36 L) was added to the vessel and the stirred mixture was cooled to 10°C. A solution of cold (5-10°C) 1M aqueous sodium hydroxide solution (16 L) and cold (5-10°C) 50% aqueous sodium hydroxide solution (2.6 L) were
- 25 added at 10°C and the temperature rose to 14°C. The resulting mixture was stirred for 15 minutes at <14°C and then the lower aqueous layer separated off.

- The batch was concentrated *in vacuo* to ~20 L volume and
- 30 then a mixture of ethyl acetate (35 L) and ethanol (5 L) was fed in while maintaining the volume at ~20 L. At the end of this distillation the KF was 9160 mgml⁻¹. The batch was solvent switched to ethyl acetate by "feeding and bleeding" ethyl acetate (40 L total). At the end of this

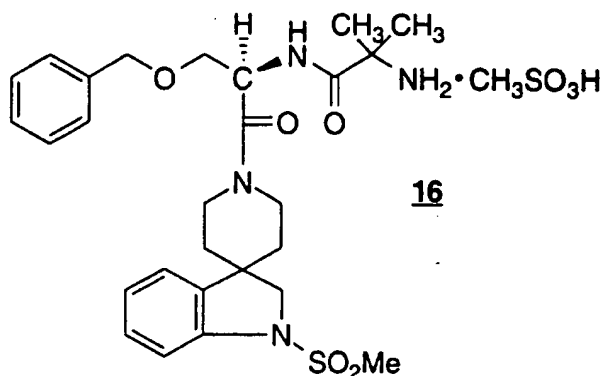
- 57 -

distillation, KF was 446 mgml^{-1} . The batch was diluted with ethyl acetate (10 L).

5 Darco G-60 (900 g) was added to the hazy mixture. This was rinsed in with ethyl acetate (6 L). This mixture was stirred at room temperature overnight. Next day, Solka Floc™ (0.5 kg) was added to the stirred batch in the vessel and then Solka Floc™ (2.0 kg) was stirred in a little ethyl acetate and loaded into an Estrella filter. The excess solvent was pumped away through a Sparkler in-line filter containing a 10μ cartridge. The slurry was transferred from the vessel through a filter
10 using a pump and then through another filter to 2 x 40 L stainless steel bins. Visual inspection showed the liquors to be clear and clean. The vessel was rinsed with ethyl acetate (22 L) and this was used to rinse through the route outlined above to the stainless steel cans. The contents of both cans was transferred into a reaction vessel and the solution was
15 mixed thoroughly.

The batch (58 L) had a KF of 2950 mgml^{-1} and so was redried by concentrating *in vacuo* to 20-25 L volume. The batch was diluted to 46 L volume (dipstick) by the addition of ethyl acetate (25 L). The KF was 363 mgml^{-1} . The batch was diluted to 62 L volume by the
20 addition of ethyl acetate (17 L) and was used for the final stage of the process.

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EXAMPLE 15A

Spiro[3H-indole-3,4'-piperdin]-1'-ylcarbonyl]-2-(phenylmethoxy)ethyl]-2-amino-2-methylpropanamide Methanesulfonate (16)

5

Materials:

	Amine (15)	2340 g (4.43 moles)
	Methane sulfonic acid (MsOH)	316 mL (4.88 moles)
	EtOAc	60 L
10	EtOH	4.8 L
	8% EtOH in EtOAc	20 L

The volume of the solution of 15 from the previous step was adjusted to 60 L with ethyl acetate and EtOH (4.8 L) was added. The

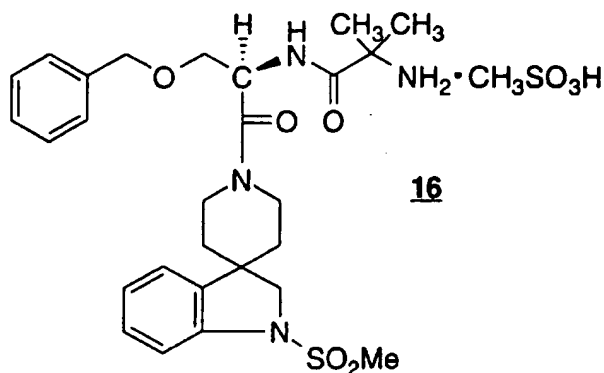
15 MsOH (316 mL) was added in 3 L of EtOAc at 45°C. To the deep red homogeneous solution was added 496 g of the title compound Form I seed (10% seed based on the weight of the free amine was employed). The temperature rose to ca. 48°C and the reaction was aged at 52°C for 1.5 hours. Analysis indicated complete conversion to the title compound

20 (Form I). (At less than 10% seed longer age (> 3 hours) was required). The slurry was allowed to cool to 20°C overnight and was filtered in a centrifuge under N₂. The cake was washed with 20 L of 8% EtOH in EtOAc. N₂ is essential during filtration because the wet crystals are very hygroscopic. The batch was dried at 35°C under vacuum to afford 2.7Kg

25 (56% overall yield) of the title compound (Form I) (99.9 A% purity; < 0.1% enantiomer).

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The conversion of Form II to Form I is also accomplished where the salt is formed in EtOAc-EtOH by addition of MsOH as above and the initial solution of the salt (at 55°C) is cooled to 45°C. Crystals start appearing at that temperature and the slurry becomes thicker with time. The temperature is then raised to 51°C and the slurry is aged overnight. Complete conversion to Form I of **16** should be expected. This procedure may also be employed to prepare seed crystals of Form I of **16**.

EXAMPLE 15B

Spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide Methanesulfonate (**16**)

Materials:

15	Amine (15)	3.1 kg (5.86 mol)
	Methanesulfonic acid	620 g (6.45 mol)
	Ethyl acetate	37 L
	Absolute ethanol	8.7 L
20	Spiro[3H-indole-3,4'-piperdin]-1'-yl)-carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate (Form I)	70 g (0.11 mol)

Absolute ethanol (6.4 L) was added to the solution of the amine (**15**) (3.1 kg) in ethyl acetate (total volume ~62 L) in a reaction vessel. The batch was warmed to 50°C and a solution of methanesulfonic

- 60 -

acid (620 g, 412 ml, 1.1 equivs.) in ethyl acetate (11 L) was added over ~5 minutes at 50-54°C. The batch was seeded with spiro[3H-indole-3,4'-piperdin]-1'-yl)-carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate (Form I) (70 g) and the resulting
5 slurry was stirred and heated at 55°C under nitrogen atmosphere overnight.

The next day, the slurry was cooled to 15-20°C, held for 2 hours and then dropped to the 50 cm polypropylene filter under nitrogen atmosphere. The solid product was washed with a mixture of absolute
10 ethanol (2.3 L) in ethyl acetate (26 L). The white, solid product was dug off and dried in an Apex oven *in vacuo* at 35°C for an appropriate time (approx. two days). The dried spiro[3H-indole-3,4'-piperdin]-1'-yl)-carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate (3.352 kg) was sieved using a Jackson-Crockatt sieve
15 to give 3.347 kg (including seed, 70 g) } yield = 3.277 kg.

Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide methanesulfonate is an anhydrous polymorph characterized by the following properties:
20 a melting point of 168-171°C and solubility in isopropanol of 4.6 mg/mL.

The DSC curve for Form I of N-[1(R)-[(1,2-dihydro-1-methanesulfonyl-spiro[3H-indole-3,4'-piperdin]-1'-yl)carbonyl]-2-(phenylmethyl-oxy)ethyl]-2-amino-2-methylpropanamide
25 methanesulfonate at 10 °C/min in an open cup under nitrogen flow exhibits a single endotherm, due to melting, with a peak temperature of about 180°C and an extrapolated onset temperature (melting point) of about 170°C with an associated heat of approximately 53 J/g.

Form I was characterized by an X-ray powder diffraction
30 pattern with reflections at approximately: 6.5, 14.7, 16.9, 17.1, 17.9, 19.5, 21.1, 21.7, and 22.0° (2 theta). Data collected using a Philips APD3720 Automated Powder Diffraction instrument with copper K α radiation. Measurements were made from 2° to 40° (2 theta) with the sample maintained at ambient room temperature.

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HPLC Conditions:

LC Retention times on Zorbax RX-C8 (4.6 mm x 25 cm),

 $\lambda = 210$ nm, flow rate = 1.5 ml/min.

- 5 Compound 1: 60:40 CH₃CN-H₂O (1% H₃PO₄) RT = 5.0 min
 Compound 1b: 35:65 CH₃CN-H₂O (0.1 w % NH₄OAc) RT = 6.2 min.
 Compound 10: 60:40 CH₃CN-H₂O (0.1 H₃PO₄) RT = 2.9 min.
 Compound 11: 60:40 CH₃CN-H₂O (0.1% H₃PO₄) RT = 5.4 min.
 Compound 12: 40:60 CH₃CN-H₂O [pH 5.25 NaH₂PO₄ (6.9 g/L of H₂O)
 10 (adjust pH with NaOH)] RT = 5.6 min
 Compound 14: 60:40% CH₃CN-H₂O (0.1% H₃PO₄) RT = 4.65 min
 Compound 15: 40:60% CH₃CN-H₂O [pH = 5.25 NaH₂PO₄ (6.9 g/L
 of H₂O)] adjust pH with NaOH) RT = 4.9 min

- 15 LC Retention times on Zorbax RX-C8 (4.6 mm x 25 cm),

 $\lambda = 210$ nm, flow rate = 1.2 ml/min, column temperature = 48°C

Solvent A = 0.05% Phosphoric acid + 0.01% Triethylamine in water

Solvent B = Acetonitrile

Gradient system:

20	<u>Time</u>	<u>% A</u>	<u>% B</u>
	0 min	95	5
	35 min	10	90
	38 min	95	5
	40 min	95	5

25

Retention time (mins)

	Compound 1	25.2
	Compound 1b	8.5
	Compound 10	20.5
30	Compound 11	26.3
	Compound 12	14.8
	Compound 14	25.6
	Compound 15	15.7

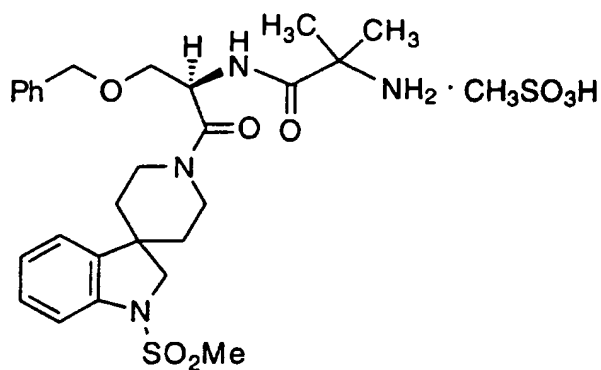
- 62 -

While the invention has been described and illustrated with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various adaptations, changes, modifications, substitutions, deletions, or additions of procedures and protocols may be made without departing from the spirit and scope of the invention. For example, reaction conditions other than the particular conditions as set forth herein above may be applicable as a consequence of variations in the reagents or methodology to prepare the compounds from the processes of the invention indicated above. Likewise, the specific reactivity of starting materials may vary according to and depending upon the particular substituents present or the conditions of manufacture, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended, therefore, that the invention be defined by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

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WHAT IS CLAIMED IS:

1. A process for the preparation of a compound of formula V:

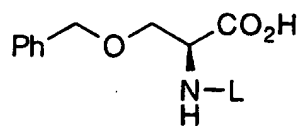


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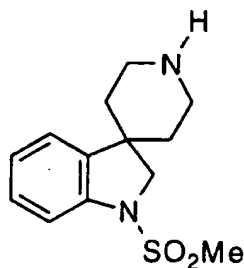
V

which comprises:

(1) coupling an amino acid of the formula:



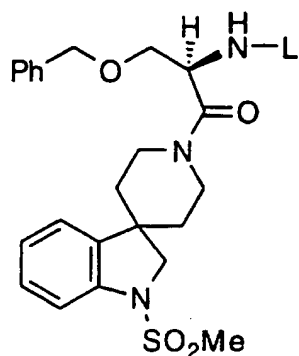
10 with a compound of the formula :



in the presence of a first acid activating agent in a first inert solvent in the

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presence of a first catalytic agent to give a compound of formula I:

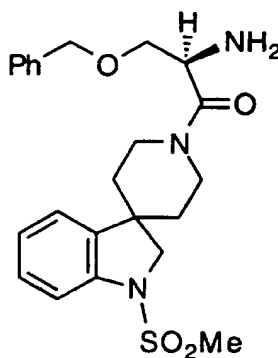


I

wherein L is an amino protecting group, followed by:

5

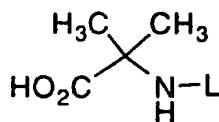
(2) reacting the compound of the formula I with a first amino deprotecting agent to give a compound of formula II:



II

10 followed by:

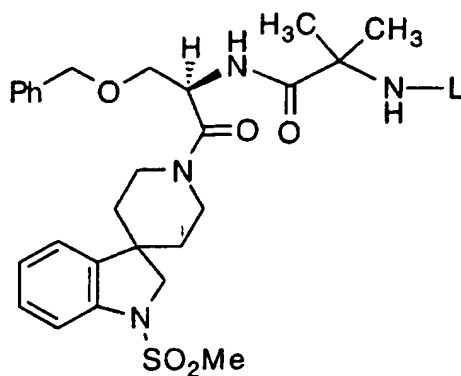
(3) coupling an amino acid of the formula:



wherein L is an amino protecting group, with the compound of

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formula II in the presence of a second acid activating agent in a second inert solvent in the presence of a second catalytic agent, to give a compound of the formula III:

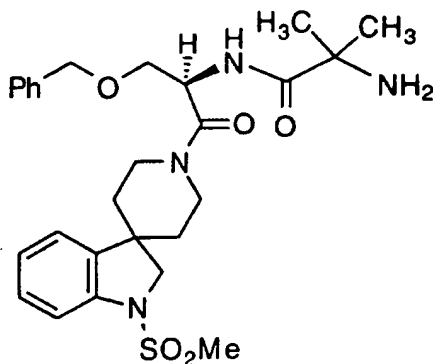


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III

wherein L is an amino protecting group,
followed by:

- (4) reacting the compound of the formula III with a second amino
10 deprotecting agent to give a compound of the formula IV, or a
pharmaceutically acceptable salt thereof:



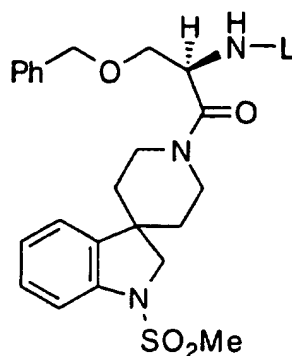
IV

followed by:

- 15 reacting the compound of the formula IV with methanesulfonic acid to
give the compound of formula V.

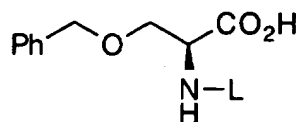
- 66 -

2. A process for the preparation of a compound of formula I:

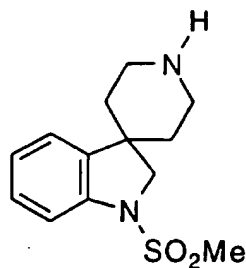


I

5 wherein L is an amino protecting group, by coupling an amino acid of the formula:



with a compound of the formula :



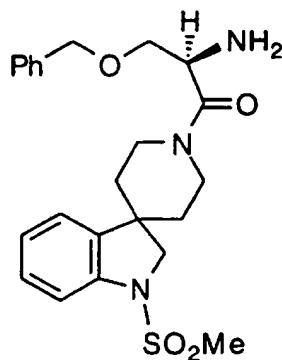
10 in the presence of an acid activating agent in an inert solvent in the presence of a catalytic agent, to give the compound of formula I.

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3. The process of Claim 2 wherein the acid activating agent is DCC.
4. The process of Claim 2 wherein the catalytic agent
5 is HOBT.
5. The process of Claim 2 wherein the solvent is selected from the group consisting of:
acetonitrile; isopropyl acetate; ethyl acetate; propionitrile;
10 water; chlorinated hydrocarbons such as dichloromethane, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene, ortho-dichlorobenzene; benzene; toluene; xylenes; and the like; and mixtures thereof.
6. The process of Claim 5 wherein the solvent is selected
15 from the group consisting of:
isopropyl acetate:water; and acetonitrile.
7. The process of Claim 2 wherein the temperature of the
20 reaction is between 20 and 35°C.
8. The process of Claim 2 wherein the compound of formula I, the amino protecting group is selected from:
t-butoxy-carbonyl.
- 25 9. The process of Claim 2 which is conducted *in situ* without isolation of the compound of formula I following its preparation.

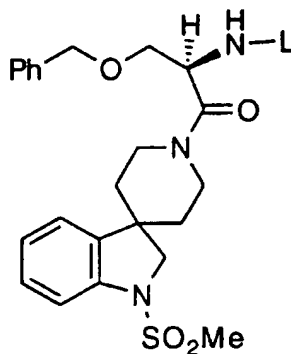
- 68 -

10. A process for the preparation of a compound of the formula II:



II

5 which comprises reacting a compound of the formula I:



I

wherein L is an amino protecting group, with an amino deprotecting agent to give the compound of formula II.

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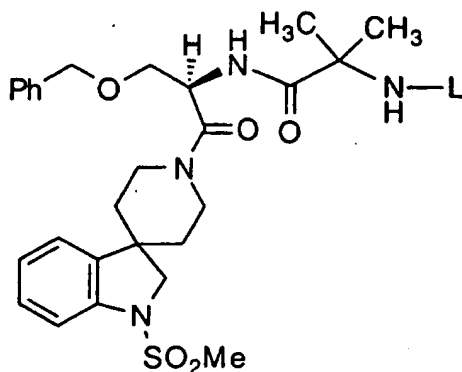
11. The process of Claim 10 wherein the compound of formula I, the amino protecting group is selected from:
t-butoxy-carbonyl.

5 12. The process of Claim 10, wherein the amino deprotecting agent is methanesulfonic acid.

10 13. The process of Claim 10 which is conducted *in situ* without isolation of the compound of formula II following its preparation.

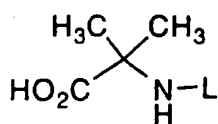
- 70 -

14. A process for the preparation of a compound of the formula III:

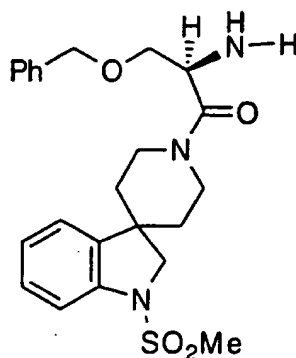


III

5 wherein L is an amino protecting group, by coupling an amino acid of the formula:



wherein L is an amino protecting group, with a compound of the formula II:



II

10

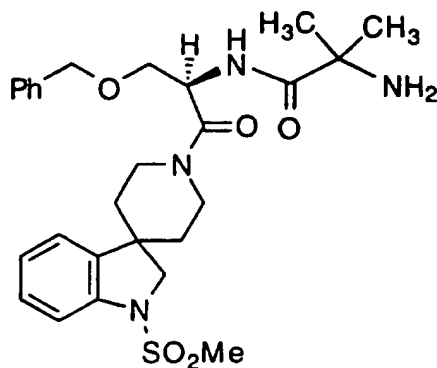
in the presence of an acid activating agent in an inert solvent in the presence of a catalytic agent, to give the compound of formula III.

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15. The process of Claim 14 wherein the acid activating agent is DCC.
- 5 16. The process of Claim 14 wherein the catalytic agent is HOBT.
17. The process of Claim 14 wherein the solvent is isopropyl acetate:water.
- 10 18. The process of Claim 17 wherein the solvent isopropyl acetate:water is in a ratio of approximately 40:60 to 60:40 (by volume).
- 15 19. The process of Claim 14 wherein the temperature of the reaction is between 20 and 35°C.
- 20 20. The process of Claim 14 wherein the compound of formula III, the amino protecting group is selected from:
t-butoxy-carbonyl.
21. The process of Claim 14 which is conducted *in situ* without isolation of the compound of formula III following its preparation.

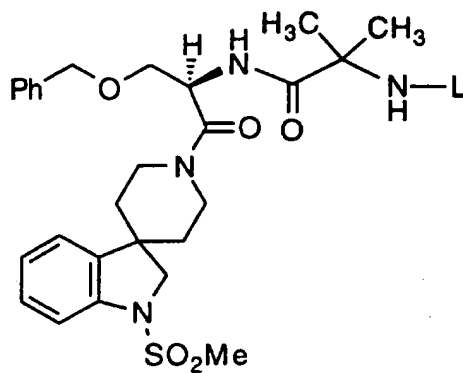
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22. A process for the preparation of a compound of the formula IV, or a pharmaceutically acceptable salt thereof:



IV

5 which comprises reacting a compound of the formula III:



III

wherein L is an amino protecting group, with an amino deprotecting agent to give the compound of formula IV.

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23. The process of Claim 22 wherein the compound of formula III, the amino protecting group is selected from:
t-butoxy-carbonyl.

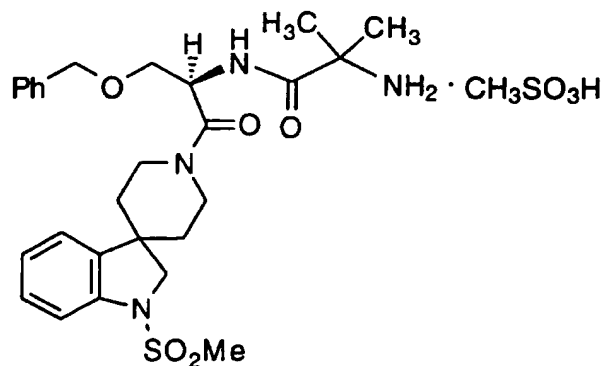
5 24. The process of Claim 22, wherein the amino deprotecting agent is methanesulfonic acid.

25. The process of Claim 22 which is conducted in a solution comprising ethanol.

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26. The process of Claim 22 which is conducted *in situ* without isolation of the compound of formula IV following its preparation.

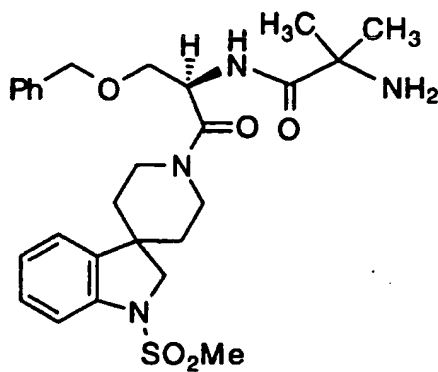
15 27. A process for the preparation of a compound of the formula V:



V

which comprises reacting a compound of the formula IV:

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IV

with methanesulfonic acid to give the compound of formula V.

- 5 28. The process of Claim 27 which is conducted in a solution comprising ethyl acetate and ethanol.

INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/US 96/16954

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D471/10 A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 13696 A (MERCK & CO INC ;CHEN MENG HSIN (US); JOHNSTON DAVID B R (US); NARG) 23 June 1994 cited in the application see Schemes see examples 18,19,55 -----	1-28

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

3 February 1997

Date of mailing of the international search report

12.02.97

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Bosma, P

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/16954

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9413696	23-06-94	US-A- 5536716	16-07-96
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		BG-A- 99710	31-01-96
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		CN-A- 1092071	14-09-94
		CZ-A- 9501514	13-12-95
		EP-A- 0615977	21-09-94
		FI-A- 952863	09-06-95
		HU-A- 9500324	28-09-95
		HU-A- 72076	28-03-96
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		JP-A- 6263737	20-09-94
		NO-A- 952295	10-08-95
		NZ-A- 258412	29-01-97
		PL-A- 309331	02-10-95
		SI-A- 9300646	30-09-94
		SK-A- 75995	08-11-95
		AU-B- 673017	24-10-96
		AU-A- 5232193	23-06-94
		CA-A- 2110672	12-06-94
		CN-A- 1092767	28-09-94
		CZ-A- 9501515	13-12-95
		EP-A- 0662481	12-07-95
		FI-A- 952862	09-06-95
		HR-A- 931484	30-04-96
		HU-A- 73228	29-07-96
		JP-B- 2509147	19-06-96
		JP-A- 7097367	11-04-95
		NO-A- 952294	10-08-95
		PL-A- 309332	02-10-95
		SI-A- 9300647	30-09-94
		WO-A- 9419367	01-09-94
		US-A- 5578593	26-11-96
		ZA-A- 9309272	08-08-94
		ZA-A- 9309274	08-08-94